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Hereditary Hearing Loss with Nephropathy (Alport's Syndrome)

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Hereditary Hearing Loss with Nephropathy (Alport's Syndrome)

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Available English articles and abstracts relating to the syndrome of hereditary hearing loss with nephropathy have been reviewed and an analysis of the hearing pattern and otologic findings in 54 affected and unaffected family members is presented

PART I

Review of the Syndrome

The concept of nephritis occurring on a hereditary basis apparently was not introduced into the medical literature until late in the nineteenth century The earliest report that could be found by the author was by Samelsohn in 1874 (12). This German author mentioned the similar character of a familial form of a renal disease to glomerulonephritis The next year an English author Dickinson, referred to three generations of a family in which 11 out of 17 members had albuminuria and several died prematurely of Bright's disease (13) A few scattered reports making reference to a hereditary pattern in renal disease appeared in the next 25 years. Kidd in 1882 (14) Benson in 1893 (15), and Pel in 1899 (16) described a hereditary background of nephritis extending over two more generations. Attlee (17) mentioned renal disease affecting several family members simultaneously but the hereditary nature of thi familial pattern was not elucidated Aitken (18) in 1909 documented the finding of albuminuria and hematuria in several generations, and Eason and Smith in 19 4 (19), reviewed the pattern of hereditary nephritis with the addition of another family Two cases of hereditary nephritis were reported in Germany in 1906 (20).

A most remarkable study developed from a family urvey first carried out by Guthrie in 190 (1) The family was kept under surveillance for a period of 25 years at Guy's Hospital in London, and reports were made by Kendall & Hertz in 191 (22), and by Hurst in 1915 and 19 3 (23 4) The author believes that Hertz & Hurst were the same man because the first and middle names are the same Cecil Alport presented the quarter century report on the family in 1927 (25) He was the first author to comment on the hearing loss as a distinctive feature of the syndrome and Williamson in 1961 (26) suggested that his name be applied to the combination of hereditary nephritis with deafness. Williamson stated.

> Reports of this syndrome have appeared under different descriptive titles which are often rather cumbersome and, since the aetiology is still unknown, may well be inaccurate. It seems, therefore, that a case can be made for the use of an eponymous title, at least for the time being. If this is accepted, then Alport's name is the obvious choice since it was he who first described the main features of the syndrome. I suggest, therefore, that the syndrome should in future be referred to as Alport's syndrome

Alport reviewed four generations of the family and reported the case studies of 29 members. Seven males and 7 females showed evidence of nephritis and 5 of the males had died before they were 20 years of age The oldest male survivor with hearing loss and nephropathy was only 13 years old. Average life span occurred in all females except one. Seven females and 4 males were noted to have perceptive deafness. He labeled the disorder hereditary familial, congenital, hemorrhagic nephritis. Alport also made the observation that the disorder was more severe in the male yet transmitted by the female and that nerve deafness may exist in individuals who are otherwise healthy The family he reported on apparently was considered to be a medical oddity and of little general interest, because only one mention was made of hereditary nephritis over the next 25 years. This was a report by Rinloff (27) in the United States in 1939 and it apparently represents the first sugges-

scopic evaluations. When conductive losses were demonstrated masked bone conduction was utilized to obtain a reasonably accurate pattern of the sensorineural reserve

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auditory disease related to such causes as drugs and infection.

Sohar was the first investigator to recognize the fact that certain ophthalmological defects were present in patients with hereditary deafness and nephropathy. He reported first from Israel in 1954 (35), and later in the United States (36) on a Jewish family. Four sons of the propositus were noted to have severe nephropathy with two having associated spherophakia and the two others posterior cataracts, apparently congenital. After Sohar's report other authors recorded eye abnormalities related to the lens, particularly lenticonus (36, 37). An increased incidence of myopia (1), cases of nystagmus (38), fundus changes (39, 40), dyschromatopsia (39) and strabismus (40) have been reported but some of these may represent complications of a chronic renal and metabolic state. The association of ocular abnormalities is generally considered part of the syndrome now. Further discussion of the eye changes is presented later but the author encountered very few eye abnormalities in the family members examined by him.

Perhaps malformations of the urinary tract should also be included as part of the syndrome since structural abnormalities have been reported by a number of authors who summarized cases or carried out family surveys (2, 43, 44, 52, 91, 93, 94). The association of genitourinary tract abnormalities in general, however, with otologic disorders is well documented in the medical literature (107, 108) and there may well be nothing specific about the association of hereditary nephritis with malformations of the genitourinary tract.

Genetics

A very detailed genetic evaluation of the syndrome and proposals to explain the mode of inheritance was recorded by Cohen and his fellow workers in 1961 (49). They tabulated four suggested inheritance patterns which included the following: 1) Partial sex-linked dominant, 2) autosomal dominant, 3) intrauterine infection or transplacental nephrotoxin and 4) preferential segregation and chromosomal association. In his report of three families from Greece, Tiliakos stated in 1964 that during cell reduction a crossing-over may have intervened and the X-chromosome crossed to Y-chromosome and vice-versa. Thus, a male patient may transmit the abnormal gene to the male child to which he transmits only Y-chromosomes. Tiliakos also performed karyotype examinations of female and male family members and found no abnormalities. The number of individuals examined is not known (40). Arnott suggested in 1966 that the defect for renal and ocular disease is inherited as a dominant but that manifestation of the gene is weakened by the presence of a sex-linked modifying gene of high incidence in the general population (42). Peters mentioned the possibility of there being one abnormal gene located in the X-chromosome (86).

Genetic delineation of the syndrome cannot be easily explained because both males and females manifest all components of the disease yet it is exceedingly more aggressive in some male siblings than others. Furthermore, the disease may be seemingly absent at birth yet become manifest in the second decade with rapid demise in the male and low-grade subclinical nephropathy and sensorineural hearing loss in the female. In addition, females may carry the trait for renal disease yet show no abnormalities on urinalysis. Pedigree documentations have been very extensive in some instances and investigators wishing to study the genetic patterns of this disease or of sex linked disorders in general should examine in detail the reports of Perkoff, Stevens, Shaw & Glover, Alport, Dubach & Minder, Tiliakos,

Table I *Authors reporting on the syndrome during the past one hundred years*

1800's	*Early 1900's*	*1920's*	*1930's*	*1950's*
Samelsohn	Guthrie	Alport	Rinkoff	Perkoff
Pel	Kendall	Hurst		Reyersbach
Kidd	Aitken	Eason		Sohar
Benson	Atlee	Hurst		Sturtz
Dickinson	Frolich			Goldbloom
	Hurst			Hamburger
1960's				Perkoff
				Nieth
Wallace	Kopelman	Holboth	Perkoff	Goldbloom
Marie	Ohlsson	Chappel	Perkoff	Graham
Whalen	Dancek	Minder	Perkoff	Mass. Gen. Case Records
Williamson	Wagner	Winter	Perrin	Morin
Reubi	Mamou	Krickstein	Cassady	Klotz
Schriver	Obertier	Zuzuki	Dalla Rosa	Poli
Dubach	Burguet	Peters	Kenderklin	Robin
Junod	Flower	Bartel	Nichol	Stephens
Schafer	Arenberg	Guerrier	Neal	Graham
Rosenkranz	Berard	Hurriet	Nieth	Russell
Rosenkranz	Beickert	Loken	Tyler	Hirsch
Johnson	Chaptal	Minder	Oropeza	Sohar
Bunge	Cross	Cassady	Pasternack	Sturtz
Tiliakos	Jarrau	Mettler	Kouvalainen	Hilson
Voulgaridis	Fleury	Perkoff	Shafer	Short
Gialafus	Arnott	Cohen	Schriver	
Dubach	Berlyne	Perkoff	Van Buchem	
Lemoyne	Mulrow	Shaw	Beetstra	
O'Pitz	Schriver	Graham	Kuhn	
Braun	Schriver		Wood	
Taylor	Jordan		Holz	

tion of the syndrome in America Rinkoff summarized the fatal cases of three brothers and reviewed the literature on hereditary nephritis up until that time He made no mention of hearing loss and postulated that the disease developed from a decreased renal resistance to inflammation and from hypersensitivity phenomena.

Perkoff and his fellow investigators spent over 10 years studying two Mormon families and published several reports of their clinical genetic and pathological findings (7 28 29 30 31 32 33) They documented the significance of white blood cells and bacteria in the urine of family members as well as the hematuria related to glomerular disease Their initial study was actually entitled Hereditary Interstitial Pyelonephritis" and it was apparently in the light of prolonged follow up that they realized the hereditary pyelonephritis in their patients was a form of the disorder described by Alport and reviewed by Rinkoff They concluded that the associated infection was somehow related to a local renal metabolic abnormality of unidentified nature These studies first reported in 1951 probably represent the initial modern studies of the syndrome

Following the revitalization of interest in hereditary deafness and nephropathy by Perkoff and his group numerous studies were initiated throughout the world and reports began to appear from Canada, France Germany Italy Israel Greece Switzerland and Japan (34–106) A list has been prepared showing authors reporting on the syndrome in the past one hundred years (Table I) Aside from the papers relating to genetics, ophthalmologic changes pathology and metabolism which are documented later these are reviews of families with the syndrome and case reports. The disease pattern is extremely variable and may include clinical pictures resembling various types of renal and auditory diseases The author has attempted to prepare a list of reports dealing basically with familial hereditary deafness and nephropathy but not covering renal and

auditory disease related to such causes as drugs and infection.

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Graham Robin, and Cohen The family studies of these authors cover many *generations* and in the case of Alport extend over periods as long as twenty five years A review of records from Switzerland permitted Dubach & Minder (8 57 74) to chart the pedigree of eight generations dating back to 1730 Other detailed geneologies are under continual surveillance such as those of Perkoff (32) and Sturz & Burke (93) and with the use of computer recording and mass screening devices more complete information relating to details of chromosome patterns, metabolism hearing loss and minor anomalies can be assembled

In 1961 Shaw & Glover (99) suggested that the trait for renal dysfunction and hearing loss is inherited as an autosomal dominant with nonrandom segregation and preferential association of the autosome carrying the gene with the X-chromosome Using a study of five families Cohen and his co-workers further supported this mode of inheritance and documented how this mechanism would explain the observed disorders They studied 339 members of four generations and concluded that the syndrome is not inherited as a partially sex linked dominant or as an autosomal dominant trait giving classical mendelian segregation ratios They stated that the trait is definitely inherited as an autosomal dominant and that the chromosome in which the defective gene resides may be preferentially selected in association with the X-chromosome. The explanation is best understood by quoting Cohen's report from the American Journal of Human Genetics. "During oogenesis in affected females, the autosome bearing the mutant gene may generally pass into the primary oocyte rather than into the polar body The defective gene would, more often than not, be incorporated in the functional ovum which would lead to an exess of affected offspring of both sexes. During spermatogenesis in affected males, the association of the X-chromosome and the trait bearing autosome would cause the two chromosomes to move together preferentially but at random to either pole leading to an excess of affected daughters and normal sons" (49)

Authors reporting on the syndrome should probably test this hypothesis using their genetic data or perhaps test one of the alternatives to see which best explains the observed number of disorders. Certainly even more valuable statistics might be collected if prospective studies aimed at predicting the affected individuals could be set up

Renal Pathology

The specific kidney disorder has been variously catalogued during the past century in a number of ways Autopsy and renal biopsy information have documented patterns consistent with glomerulonephritis pyelonephritis and interstitial nephritis (33 36 70 101 102) In renal biopsies of a case followed for ten years by Van Buchem the primary disorder has been considered to be in the distal tubules with progressive involvement of other renal structures as the patient grows older (101) Most cases of the classic syndrome have demonstrated certain findings of glomerulonephritis on biopsy or post-mortem examinations In addition a recording of renal foam cells is often made and Perkoff noted these frequently in specimens obtained from his family members (7) The lipidcontaining foam cells were noted by other observers also (6, 50 52 60), and were thought at one time to be pathognomonic of the hereditary syndrome and specific enough to permit pathologists to confirm the diagnosis with renal biopsy However Whalen who examined some of the patients reported in Part II by the author reviewed 105 consecutive cases of pyelonephritis and

105 consecutive cases of glomerulonephritis and demonstrated that the foam cells were present in disease states having no basis in heredity and, therefore, were not pathognomonic (102). Whalen also noted that the foam cells probably contained fat, mucopolysaccharides, phospholipids, cholesterol and phosphatides. A pathological picture resembling pyelonephritis can definitely be found in some patients with the syndrome (28, 60, 70, 87) but this may represent a body stress effect with lowered resistance in a target organ as suggested by other authors (1). Generally speaking, the pathologic diagnosis in young individuals has been glomerulonephritis while that in older patients has usually been pyelonephritis.

Various renal symptoms and signs are seen in the disease with hematuria being the initial and principal finding. It has been documented in a ten day old infant (73) and may occur insidiously and subclinically throughout life. At various stages of the disease proteinuria, pyuria, cylindruria and amino-aciduria may occur (67, 74, 94, 95, 96, 97, 106). Urine cultures are usually negative. The documentation of renal dysfunction can be quite difficult and some sort of standard is needed to classify the particular patient as being involved or free of the syndrome. One survey used only fresh centrifuged urine with three red cells and/or five white cells per HPF or an abnormal Addis count as evidence of renal dysfunction. Isolated proteinuria was not considered evidence of disease (1). Perhaps strict criteria such as this would permit more accurate identification of affected relatives of diagnosed patients.

In 1966, a classic paper detailing the pathology in nine autopsy and seven renal biopsy cases was presented by Krickstein, Gloor & Balogh (68). In this scholarly work the authors described in great detail the pathological picture of hereditary nephritis and indicated that there were two major facets to the disease. First, hereditary nephritis usually shows a combination of certain features of glomerulonephritis, pyelonephritis and interstitial nephritis but also lacks certain characteristics of each of these forms of nephritis. A "mixed" type of nephritis thus seems to be the distinctive feature of the hereditary syndrome somewhat analogous to the mixed tumor of the parotid. Secondly, according to Krickstein, the occurrence of foam cells in a practically specific distribution in the lower renal cortex helps to establish the unique histological appearance. Although the foam cells are seen in other types of nephritis, no other type shows them with the same consistency, quantity and distribution as does hereditary nephritis. They state that the foam cells are derived from tubular epithelial cells which have undergone degenerative changes.

The pathogenesis of the renal disorder remains in doubt, but several theories have been proposed by Krickstein and his associates. There may be a family susceptibility to beta hemolytic streptococci, or a structural embryopathy occurring at the time of formation of the renal and auditory systems may exist. An inherited defect of fat metabolism may exist, but there is no evidence of this in other organs. They favor the idea that a genetically controlled defect in an enzyme system common to the renal, auditory and ocular tissues is present. This defect renders these structures susceptible to harmful affects of various types and may lead to the accumulation of a toxic metabolic product with slowly progressive damage to the organ systems involved. This common susceptibility is seen in normal individuals from the ototoxic and nephrotoxic affects of certain drugs like kanamycin. This inborn metabolic error of metabolism would explain the normal renal biopsy reports seen early in the disease with progression to nephritis later (101). The renal biopsy studies of Krickstein indicate, however, that foam cells may be picked up early in the disease before other changes have become pronounced and thus alert the pathologist and the clinician to the likelihood of hereditary nephritis.

Schriver in 1961 (95, 97) and Schriver, Efron and Schaler in 1964 (96) reported

Graham, Robin, and Cohen The family studies of these authors cover many generations and in the case of Alport extend over periods as long as twenty five years A review of records from Switzerland permitted Dubach & Minder (8 57 74) to chart the pedigree of eight generations dating back to 1730 Other detailed geneologies are under continual surveillance such as those of Perkoff (32) and Sturz & Burke (93) and with the use of computer recording and mass screening devices more complete information relating to details of chromosome patterns, metabolism hearing loss and minor anomalies can be assembled.

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Various renal symptoms and signs are seen in the disease with hematuria being the initial and principal finding. It has been documented in a ten day old infant (73) and may occur insidiously and subclinically throughout life. At various stages of the disease proteinuria, pyuria, cylindruria and amino-aciduria may occur (67 74 94 95 96, 97 106) Urine cultures are usually negative The documentation of renal dysfunction can be quite difficult and some sort of standard is needed to classify the particular patient as being involved or free of the syndrome One survey used only fresh centrifuged urine with three red cells and or five white cells per HPF or an abnormal Addis count as evidence of renal dysfunction Isolated proteinuria was not considered evidence of disease (1) Perhaps strict criteria such as this would permit more accurate identification of affected relatives of diagnosed patients

In 1966, a classic paper detailing the pathology in nine autopsy and seven renal biopsy cases was presented by Krickstein, Gloor & Balogh (68) In this scholarly work the authors described in great detail the pathological picture of hereditary nephritis and indicated that there were two major facets to the disease. First, hereditary nephritis usually shows a combination of certain features of glomerulonephritis, pyelonephritis and interstitial nephritis but also lacks certain characteristics of each of these forms of nephritis. A mixed" type of nephritis thus seems to be the distinctive feature of the hereditary syndrome somewhat analogous to the mixed tumor of the parotid. Secondly according to Krickstein, the occurrence of foam cells in a practically specific distribution in the lower renal cortex helps to establish the unique histological appearance Although the foam cells are seen in other types of nephritis, no other type shows them with the same consistency quantity and distribution as does hereditary nephritis. They state that the foam cells are derived from tubular epithelial cells which have undergone degenerative changes.

The pathogenesis of the renal disorder remains in doubt, but several theories have been proposed by Krickstein and his associates. There may be a family susceptibility to beta-hemolytic streptococci, or a structural embryopathy occurring at the time of formation of the renal and auditory systems may exist. An inherited defect of fat metabolism may exist, but there is no evidence of this in other organs. They favor the idea that a genetically controlled defect in an enzyme system common to the renal, auditory and ocular tissues is present. This defect renders these structures susceptible to harmful affects of various types and may lead to the accumulation of a toxic metabolic product with slowly progressive damage to the organ systems involved. This common susceptibility is seen in normal individuals from the ototoxic and nephrotoxic affects of certain drugs like kanamycin. This inborn metabolic error of metabolism would explain the normal renal biopsy reports seen early in the disease with progression to nephritis later (101) The renal biopsy studies of Krickstein indicate, however that foam cells may be picked up early in the disease before other changes have become pronounced and thus alert the pathologist and the clinician to the likelihood of hereditary nephritis

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that hereditary nephritis is associated with hyperprolinemia and hyperaminoaciduria A common transport system for renal tubular re absorption is shared by the amino acids proline hydroxyproline and glycine according to these investigators This metabolic defect has been studied by other investigators particularly Minder & Dubach (8 57 66 67 74 82, 106) They observed hyperprolinemia in six of 21 siblings who were related to a known family with nephropathy Hyperaminoaciduria was found in eight of the 21 siblings Methyl histidine excretion in the urine of family mem bers was also noted by these authors They interpreted these findings as a mute" factor however probably indicating inheritance from one parent that occurred coincidentally in con junction with the abnormal gene for ne phropathy from the other parent. One author has also reported amino-acidurias involving alanine glutamic acid histidine and threonine in the syndrome but no other confirmation of this could be located by the author (82)

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A complete histological study of the inner ear changes in hereditary hearing loss and ne phropathy was not reported until late in 1968 (5) Winter and his co-workers obtained the temporal bones on a 22 year-old white male who died with the syndrome and who gave a history of hearing loss extending back to age 12 and of albuminuria documented at age 5 years The patient was approaching a terminal state and had severe loss of hearing for middle frequencies primarily Bilateral anterior len ticonus was found Winter was struck by the minimal changes on the temporal bone sec tions The tectorial membrane organ of Corti and Reissner s membrane all were normal There was vesicle formation in the spiral liga ment of each basal turn and some degenera tion of the spiral nerves supplying the basal turns with approximately 50% reduction in basal turn spiral ganglion cells Similar vesicle formation was noted under each utricular macule Both temporal bones were essentially identical as were the audiograms recorded nine months prior to death

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Audiometric Abnormalities

An otolaryngologist, Mr I Gay French, examined two of the cases from Alport's family in 1916 Progressive hearing change was noted in one case and both were diagnosed as having nerve deafness. Alport also noted that the hearing loss was present in some patients who did not manifest nephritis (25) Throughout the family reports in the last forty years sensorineural hearing loss has been the hallmark of the hearing component. In the families studied early by Perkoff, 14 out of 59 members were reported as having abnormal audiograms (28). A separate report on these patients by Stevens and Perkoff indicates that the hearing deficit was found only in males and varied from high tone loss to a moderately severe deafness. They stated that two small groups showed a high tone loss or a dip at 4096 respectively (33) In a later report on these patients 86 audiographic studies were performed. The audiograms of 20 males and one female showed high tone deafness. Two males and four females had abnormalities in the high tone range of doubtful significance The conditions under which these tests were made is not known nor do they record their criteria for determining deafness. Out of a total of 168 family members examined by Perkoff, 22 males and 44 females had no indication of renal or ear disease. Twenty-three patients (two males) had normal hearing with definite renal disease and only six males had hearing loss with normal kidney function. It was his observation that the nerve deafness usually manifested itself in males as the renal disease became severer (7)

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and states that if the anterior capsule is weak a lenticonus develops but if it is deficient, a type of anterior polar cataract is formed. Thus, these two abnormalities seen in the hearing loss and nephropathy syndrome probably represent two stages of the same defect. The occurrence of spherophakia may also be a further stage of the same process since it is presumably due to a defect in the zonule (37)

Junod (65) and Ohlsson (82) in 1963 mention myopia as being a part of the ophthalmologic defects, and this seems to have been confirmed by a six year survey of 476 family members reported in 1965 (1) Tiliakos in his review of a Greek family noted one case of strabismus and one of pigmented retinitis. No recordings of lenticonus were noted (40) White punctate lesions in the fundus were recorded by Bunge in 1965 (39) It is likely that pigmentary changes noted in the syndrome are incidental. Cataracts are mentioned regularly in the family reviews listed in Table I. Dyschromatopsia was recorded in a Belgium family in 1966 (48) Perrin in 1964 did very careful ophthalmologic screening of 238 family members. He recorded alterations in the shape of the lens such as micospherophakia, anterior and posterior lenticonus, alterations in the transparency of the lens, anterior polar cataracts, subcapsular anterior or posterior cataracts nuclear cataracts and thickening of the central and anterior layers of the cornea accompanied by nystagmus. He recommended that slit-lamp and fundus examination be performed on family members (38) Another author commented on retrocorneal spindle shaped deposits in two cases of the syndrome (50) The latest eye report by Arnott in the British Journal of Ophthalmology again describes the finding of anterior lenticonus (42) The classic case studied histologically by Winter also had lenticonus (5)

Apparently fully-developed or advanced cases of the disease will manifest lens abnormalities and these may be related more to the chronic renal disease than to a hereditary anomaly If a genetic metabolic error does predispose the lens to poor fluid exchange or to toxic deposition, then the defect should probably be considered primary The author could not document any instances of lenticonus in his 54 family members reported in Part II. One coloboma was noted, and several refractive errors were recorded but not investigated because of inadequate eye preparation.

fect (74) Later they concluded that the nerve deafness in the family ran about 16–20% with an incidence of renal disease of 29% (8)

Certainly young children may have the sensorineural hearing loss although it usually becomes manifest in the second decade in the male and later in the female One French report even mentions nerve deafness beginning in infancy (72) Modern infant audiometry does permit testing at this age with reliable results.

Sohar mentioned that recruitment was present in two of his cases with sensorineural loss in 1954 (34) Goldbloom recorded normal dorsal and ventral cochlear nuclei and tracts in his study of a case in 1957 (60) Klotz documented the air and bone conduction levels in a case studied over a two-year interval Further decline in hearing for all frequencies 256 to 8 192 cps was recorded (4) Johnson performed audiometry on several family members of a kindred in 1965 and stated that the pure tone curves were of two types. One was a descending curve with loss of hearing more severe in the high frequencies The other type manifested a drop at 4 096 cps. He did not list the number of examinations done nor the conditions of testing Speech testing and discrimination scores were recorded in twin boys age 17 and 18 One also had positive SISI scores at 500 and 2 000 cps and a Type II Bekesy tracing (2) This was strong evidence for the presence of a cochlear lesion rather than a retrocochlear one Suzuki documented a case from Japan exhibiting recruitment with normal vestibular function tests and normal petrous apex films (6)

The hearing pattern of the histologically verified case of Winter included a moderate loss for pure tones (range 30–65 dB ASA), slight discrimination loss and normal SISI scores Temporal bone pathology showed 50% loss of spiral ganglion cells in the basal turns (5)

In 1955 Poli called attention to the unusually high incidence of severe otitis media in the syndrome (85) and this finding has been recorded by other investigators (1 82) The author encountered a number of ear drum scars and distortions in his examinations reported in Part II However not a single perforation was noted. Several ear drum retractions were seen indicating Eustachian tube malfunction and negative Rinne tests were recorded for 256 and 512 cps in these patients. Perhaps greater attention should be given to recording a history of ear infections when female members with the syndrome were examined. The primary value would seem to lie in differentiating family suspects with hearing loss who might be erroneously labeled with the disease unless the conductive nature of the hearing loss was recognized As mentioned under temporal bone pathology none of the three reported histological examinations made any mention of inflammatory disease or residue in the middle ear

Ocular Abnormalities

Since the notation by Sohar in 1954 of spherophakia and cataracts, more careful attention has been paid to the identification of ophthalmologic disorders Anterior subcapsular cataracts were discovered in one family member by Goldbloom in 1957 (60) Mention was made of congenital abnormalities of the eyes in a report by Reyersbach & Butler in 1954 but no specific descriptions were given (91) Chappel mentioned lens deformities and alpha 2 globulin disturbances in his report in 1960 (51) Two detailed cases of lenticonus were described by Mettier in 1961 and one patient had a retinal detachment The detachment was considered to be incidental. Mettier describes the pathogenesis of the abnormalities,

and states that if the anterior capsule is weak a lenticonus develops but if it is deficient, a type of anterior polar cataract is formed. Thus, these two abnormalities seen in the hearing loss and nephropathy syndrome probably represent two stages of the same defect. The occurrence of spherophakia may also be a further stage of the same process since it is presumably due to a defect in the zonule (37).

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PART II

The Audiometric Survey

In 1959 particular attention was drawn to the hereditary syndrome of hearing loss and nephropathy at our medical center because of a specific interest on the part of several internists in more accurately delineating renal pathology thought to be pathognomic of the disorder (102) Once several patients with possible hereditary nephropathy were identified then a concerted effort was made to evaluate relatives who might be affected Three separate kindred who were geographically accessible to the medical center were identified over a period of three years and a survey was made to obtain urinalyses, hearing tests otologic evaluations and screening eye examinations

A large number of individuals of each kindred were contacted but only a small number could be reached for audiometric screening or persuaded to visit the hospital for specific examination There were 216 members identified in the first kindred 233 in the second, and 140 in the third Screening audiograms were taken in the field by another examiner on 92 of this total number From this group of 92 family members representing an age range of 2 to 65 years, 56 individuals visited the hospital clinics for controlled, identical audiometric testing and otologic examinations All members were encouraged to attend but many could not conveniently come therefore the selection of patients was randomized on the basis of volunteers A comparison of the screened with the examined group indicates that a valid sample of each age group volunteered for more exact testing Very few children under five years of age were contacted Audiometric results in the very young would probably be normal since other authors have indicated (1 5 7 100 101 103) that individuals affected with the full or partial syndrome have shown a progressive loss as the renal disease increases in its severity The common occurrence of serous otitis media in the age group under five years would probably complicate specific identification of hearing losses as would the testing techniques of bone conduction In addition there were only two children screened in the age group 0–4 years one 2 years old and one 4 years old thus the numerical loss from the total group studied is quite small. Table II shows a comparison of screened with examined family members

The three kindred studied all live in a single southern state and are of Anglo-Saxon descent. The families were largely settled in limited geographic areas within the state and family relationships are well-known Knowledge of the geneology permitted pedigree recordings as far back as five generations in two families and six generations in the other The pattern of the disease based on available urologic and otologic testing is recorded in Charts I II and III A clinical history of hearing impairment rather than direct testing was used by interviewers to designate some family members as being af

Table II *Number of family members screened and examined*

Age group	No. screened	No. examined
0–4 y	2	0
5–15	38	30
16–49	39	21
50 +	6	3

Table III *Audiometric findings in males (5–15 years)*

Case no.	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease[a]
35	25	15	20	10	10	10	10	10	10	5	5	5	−5	−10	Yes
21	0	5	10	10	−5	15	0	5	0	−10	10	10	10	−5	No
133	5	−5	10	10	−10	−5	−5	5	10	5	−5	5	10	5	Yes
192	10	10	15	15	10	10	10	10	5	10	10	10	10	15	No
77	15	5	5	−10	5	−5	−5	0	−5	0	15	10	20	0	Yes
24	10	15	10	10	0	10	10	10	5	10	20	20	0	20	No
22	0	0	5	5	5	−5	15	0	15	0	20	5	5	−5	No
8	5	10	5	10	5	5	5	15	10	−10	15	5	−5	−10	No
52	5	5	5	5	5	5	5	5	5	5	20	5	5	5	Yes
20	5	5	0	0	0	5	10	0	0	0	50	35	30	20	No
191	5	15	15	10	0	10	0	10	5	10	15	10	0	5	No
176	5	0	0	0	−5	0	−10	0	0	0	20	5	−10	−10	Yes
211	5	10	5	5	5	10	5	10	5	10	25	5	5		No
102	10	10	15	20	20	30	25	40	30	45	25	25	5	5	Yes
103	10	15	10	10	5	15	10	25	25	35	30	25	20	15	No
Average	6	8	6	6	3	7	6	10	8	7	18	12	7	2	
Binaural	7		6		5		8		8		15		4		

[a] Criteria and evaluation for renal disease reported elsewhere

flicted with the syndrome and, therefore, only a general idea of the pattern of inheritance can be obtained from these charts. In the affected family members the hearing loss was always insidious in onset and in many cases unknown until specific audiometry was performed.

Methods

Individuals who had been screened by audiometer (Maico portable MA2B) were requested to visit the Otolaryngology Clinic of the medical center for more controlled studies. An

Table IV *Audiometric findings in females (5–15 years)*

Case no.	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease
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190	0	10	10	10	10	10	10	10	5	10	10	10	10	10	Yes
160	5	10	10	5	10	10	10	5	5	5	5	5	5	10	Yes
161	10	5	10	5	10	5	10	15	10	10	10	10	10	10	Yes
76	20	20	5	10	10	10	10	10	10	10	10	10	5	5	Yes
67	20	25	20	40	40	55	35	60	30	20	45	45	20	20	Yes
54	5	5	5	5	5	5	5	5	10	15	40	15	20	15	
34	5	5	5	5	5	5	5	0	5	10	10	5	10	0	Yes
33	5	10	10	10	5	10	10	10	5	0	5	10	−5	0	Yes
126	5	15	10	5	5	5	5	5	5	15	10	10	5	5	No
132	5	0	5	5	5	5	5	10	5	0	5	−5	−5	5	Yes
23	5	0	10	10	10	5	−5	−10	−10	10	0	30	−5	−15	No
15	5	5	5	5	5	5	5	5	5	5	10	5	5	5	Yes
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175	0	0	5	5	8	0	5	0	0	10	20	5	5	0	Yes
Average	5	8	5	7	5	7	7	9	6	7	13	13	6	9	
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192	10	10	15	15	10	10	10	10	5	10	10	10	10	15	No
77	15	5	5	−10	5	−5	−5	0	−5	0	15	10	20	0	Yes
24	10	15	10	10	0	10	10	10	5	10	20	20	0	20	No
22	0	0	5	5	5	−5	15	0	15	0	20	5	5	−5	No
8	5	10	5	10	−5	5	5	15	10	−10	15	5	−5	−10	No
52	5	5	5	5	5	5	5	5	5	5	20	5	5	5	Yes
20	5	5	0	0	0	5	10	0	0	0	50	35	30	20	No
191	5	15	15	10	0	10	0	10	5	10	15	10	0	5	No
176	5	0	0	0	−5	0	−10	0	0	0	20	5	−10	−10	Yes
211	5	10	5	5	5	10	5	10	5	10	25	5	5		No
102	10	10	15	20	20	30	25	40	30	45	25	25	5	5	Yes
103	10	15	10	10	5	15	10	25	25	35	30	25	20	15	No
Average	6	8	6	6	3	7	6	10	8	7	18	12	7	2	
Bilateral	7		6		5		8		8		15		4		

[a] Criteria and evaluation for renal disease reported elsewhere.

flicted with the syndrome and, therefore, only a general idea of the pattern of inheritance can be obtained from these charts. In the affected family members the hearing loss was always insidious in onset and in many cases unknown until specific audiometry was performed.

Methods

Individuals who had been screened by audiometer (Maico portable MA2B) were requested to visit the Otolaryngology Clinic of the medical center for more controlled studies. An

Table IV *Audiometric findings in females (5–15 years)*

	256		512		1024		2048		4096		5792		8192		
Case no	R	L	R	L	R	L	R	L	R	L	R	L	R	L	Renal disease
177	0	0	5	0	10	5	10	5	5	10	15	10	10	−5	
190	0	10	10	10	10	10	10	10	5	10	10	10	10	10	Yes
160	5	10	10	5	10	10	10	5	5	5	5	5	5	10	Yes
161	10	5	10	5	10	5	10	15	10	10	10	10	10	10	Yes
78	20	20	5	10	10	10	10	10	10	10	10	10	5	5	Yes
67	20	25	20	40	40	55	35	60	30	20	45	45	20	20	Yes
54	5	5	5	5	−5	5	5	5	10	15	40	15	20	15	
34	5	5	5	5	5	5	5	0	5	10	10	−5	10	0	Yes
33	5	10	10	10	5	10	10	10	5	0	5	10	−5	0	Yes
126	5	15	10	5	5	5	5	5	5	15	10	10	5	5	N
132	5	0	5	5	5	5	5	−10	5	0	5	5	5	5	Yes
23	5	0	10	10	10	5	5	10	−10	−10	0	30	−5	−15	No
15	5	5	5	5	5	5	5	5	5	5	10	5	5	5	Yes
54	5	15	5	25	5	10	5	25	5	30	5	40	5	25	Yes
175	0	0	5	5	0	0	5	0	0	−10	20	5	5	0	Yes
Average	5	8	5	7	5	7	7	9	6	7	13	13	6	9	
Bilateral	6		6		6		8		6		13		8		

PART II

The Audiometric Survey

In 1959 particular attention was drawn to the hereditary syndrome of hearing loss and nephropathy at our medical center because of a specific interest on the part of several internists in more accurately delineating renal pathology thought to be pathognomic of the disorder (102) Once several patients with possible hereditary nephropathy were identified, then a concerted effort was made to evaluate relatives who might be affected Three separate kindred who were geographically accessible to the medical center were identified over a period of three years and a survey was made to obtain urinalyses hearing tests, otologic evaluations and screening eye examinations.

A large number of individuals of each kindred were contacted but only a small number could be reached for audiometric screening or persuaded to visit the hospital for specific examination There were 216 members identified in the first kindred 233 in the second, and 140 in the third Screening audiograms were taken in the field by another examiner on 92 of this total number From this group of 92 family members representing an age range of 2 to 65 years, 56 individuals visited the hospital clinics for controlled identical audiometric testing and otologic examinations. All members were encouraged to attend but many could not conveniently come, therefore the selection of patients was randomized on the basis of volunteers. A comparison of the screened with the examined group indicates that a valid sample of each age group volunteered for more exact testing Very few children under five years of age were contacted Audiometric results in the very young would probably be normal since other authors have indicated (1 5 7 100 101 103) that individuals affected with the full or partial syndrome have shown a progressive loss as the renal disease increases in its severity The common occurrence of serous otitis media in the age group under five years would probably complicate specific identification of hearing losses as would the testing techniques of bone conduction In addition, there were only two children screened in the age group 0–4 years, one 2 years old and one 4 years old thus the numerical loss from the total group studied is quite small Table II shows a comparison of screened with examined family members.

The three kindred studied all live in a single southern state and are of Anglo-Saxon descent. The families were largely settled in limited geographic areas within the state and family relationships are well-known Knowledge of the geneology permitted pedigree recordings as far back as five generations in two families and six generations in the other The pattern of the disease based on available urologic and otologic testing is recorded in Charts I II and III A clinical history of hearing impairment rather than direct testing was used by interviewers to designate some family members as being af

Table II. *Number of family members screened and examined*

Age group	No. screened	No. examined
0–4 y	2	0
5–15	38	30
16–49	39	21
50 +	6	3

Table III *Audiometric findings in males (5–15 years)*

Case no.	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease[a]
35	25	15	20	10	10	10	10	10	10	5	5	5	−5	−10	Yes
21	0	5	10	10	−5	15	0	5	0	−10	10	10	10	−5	No
133	−5	−5	−10	−10	−10	−5	−5	5	10	5	−5	5	10	5	Yes
192	10	10	15	15	10	10	10	10	5	10	10	10	10	15	No
77	15	5	5	−10	5	−5	−5	0	−5	0	15	10	20	0	Yes
34	10	15	10	10	0	10	10	10	5	10	20	20	0	20	No
22	0	0	5	5	5	−5	15	0	15	0	20	5	5	−5	No
8	5	10	5	10	−5	5	5	15	10	−10	15	5	−5	−10	No
52	5	5	5	5	5	5	5	5	5	5	20	5	5	5	Yes
20	5	5	0	0	0	5	10	0	0	0	50	35	30	20	No
191	5	15	15	10	0	10	0	10	5	10	15	10	0	5	No
176	5	0	0	0	−5	0	−10	0	0	0	20	5	10	−10	Yes
211	5	10	5	5	5	10	5	10	5	10	25	5	5		No
102	10	10	15	20	20	30	25	40	30	45	25	25	5	5	Yes
103	10	15	10	10	5	15	10	25	25	35	30	25	20	15	No
Average	6	8	6	6	3	7	6	10	8	7	18	12	7	2	
Binaural	7		6		5		8		8		15		4		

[a] Criteria and evaluation for renal disease reported elsewhere.

flicted with the syndrome and, therefore, only a general idea of the pattern of inheritance can be obtained from these charts. In the affected family members the hearing loss was always insidious in onset and in many cases unknown until specific audiometry was performed.

Methods

Individuals who had been screened by audiometer (Maico portable MA2B) were requested to visit the Otolaryngology Clinic of the medical center for more controlled studies. An

Table IV *Audiometric findings in females (5–15 years)*

Case no	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease
177	0	0	5	0	10	5	10	5	5	10	15	10	10	−5	
190	0	10	10	10	10	10	10	10	5	10	10	10	10	10	Yes
160	5	10	10	5	10	10	10	5	5	5	5	5	5	10	Yes
161	10	5	10	5	10	5	10	15	10	10	10	10	10	10	Yes
76	20	20	5	10	10	10	10	10	10	10	10	10	5	5	Yes
67	20	25	20	40	40	55	35	60	30	20	45	45	20	20	Yes
54	5	5	5	5	5	5	5	5	10	15	40	15	20	15	
34	5	5	5	5	5	5	5	0	5	10	10	−5	10	0	Yes
33	5	10	10	10	5	10	10	10	5	0	5	10	−5	0	Yes
126	5	15	10	5	5	5	5	5	5	15	10	10	5	5	No
132	5	0	5	5	5	5	5	−10	5	0	5	5	−5	5	Yes
23	5	0	10	10	10	−5	5	−10	−10	−10	0	30	−5	−15	No
15	5	5	5	5	5	5	5	5	5	5	10	5	5	5	Yes
54	5	15	5	25	5	10	5	25	5	30	5	40	5	25	Yes
175	0	0	5	5	0	0	5	0	0	−10	20	5	5	0	Yes
Average	5	8	5	7	5	7	7	9	6	7	13	13	6	9	
Binaural	6		6		6		8		6		13		8		

Table V *Audiometric findings in males (16–49 years)*

Case no	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease
75	15	15	5	10	10	10	10	5	25	15	50	50	10	-5	Yes
216	15	10	10	10	10	10	25	20	20	25	-5	30	0	15	Yes
13	15	5	5	5	15	0	15	0	15	-10	35	20	50	15	Yes
125	10	5	10	5	5	5	0	5	10	-5	10	20	5	15	Yes
14	5	5	5	5	5	5	5	20	5	10	10	15	15	10	Yes
53	0	10	0	5	0	5	0	5	5	10	10	10	10	5	Yes
Average	10	8	5	6	7	6	9	9	13	7	23	24	15	9	
Binaural	9		6		7		9		10		24		1		

initial routine otolaryngology examination was performed which included inspection of the pharynx and nasopharynx, transillumination of the frontal and maxillary sinuses, palpation of the neck and otoscopic evaluation of the external auditory canal and tympanic membrane with a pneumatic otoscope. Tuning fork testing with 256 and 512 cps steel forks was performed and results of the Rinne and Weber tests were recorded. If a difference in hearing between air and bone conducted sound was identified to either of these two forks then carefully masked bone audiometry was performed. Fork testing was performed in an *Industrial Acoustics sound room with the door* ajar Pure tone testing and bone audiometry was performed with the door closed and with an attenuation in decibels of 54 at 4800–10 KC. Unilateral airbone gaps were tested by fork with a Barany noise maker masking the opposite car Bone conduction audiometry was performed with a head-band, light weight mastoid attenuator Masking was accomplished with a saw-toothed noise-maker at 40 dB above threshold Testing was carried out with a Beltone 14–A audiometer in a two-room setting with the patient under direct observation The audiometer was calibrated according to American Standards Association 1951 levels and charts were prepared using these values as *reference standards. Frequencies between 256* and 8 192 cps were tested The same instruc

Table VI *Audiometric findings in females (16–49 years)*

Case no	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	819 R	819 L	Renal disease
134	5	5	5	5	5	10	5	10	5	10	20	10	10	10	Yes
63	10	10	10	10	10	5	10	10	10	5	5	10	5	5	No
136	5	5	5	5	5	5	5	5	5	5	5	20	5	5	Yes
36	15	15	10	10	10	10	10	10	10	10	15	20	10	10	Yes
18	0	10	10	10	10	15	40	25	-5	25	45	-5	30	25	Yes
131	5	15	-5	10	-5	0	20	0	30	10	35	40	30	20	Yes
130	10	10	5	0	15	10	25	20	15	10	20	20	20	5	Yes
148	0	0	0	-5	0	-5	0	15	10	5	30	20	25	15	No
189	10	10	5	10	0	10	10	25	10	10	40	40	10	5	Yes
82	20	20	20	5	10	10	10	5	15	5	10	5	10	5	No
173	0	10	5	0	15	15	0	10	25	25	-5	10	5	-5	Yes
65	5	5	10	10	5	0	0	15	-5	40	10	30	5	10	N
163	20	10	25	10	20	10	15	15	70	40	80	30	35	20	Yes
214	10	15	10	10	5	10	5	10	10	10	10	10	10	10	No
215	-10	-10	-10	-10	-10	-10	-10	-10	0	0	5	0	10	10	No
Average	7	9	6	5	6	6	10	11	14	14	24	19	15	10	
Binaural	8		6		6		10		14		22		12		

Table VII *Audiometric findings in one male and two females (Age 63 56 and 63 years)*

Case no.	256 R	256 L	512 R	512 L	1024 R	1024 L	2048 R	2048 L	4096 R	4096 L	5792 R	5792 L	8192 R	8192 L	Renal disease
69	10	10	20	10	−5	10	20	5	25	30	45	55	65	50	Yes
79	20	10	15	20	10	5	0	20	25	40	40	40	15	25	Yes
5	10	5	−5	5	5	−5	10	15	−5	30	70	55	40	20	Yes
Average	13	8	10	11	0	3	10	13	15	33	51	50	40	35	
Binaural	11		11		2		11		26		50		38		

tions were given to each patient and each responded with a hand signal. Test tones were presented in an ascending fashion going from silence up to threshold using a pulsed tone for both air and bone signals. Each threshold was checked by testing the next lowest intensity level at least twice. An average response was not obtained but the intensity recorded represented the response given on at least two of the three trials. A single response not reproducible at least twice, was not used as threshold.

The author attempted to obtain a hearing level which would be meaningful to physicians and audiologists encountering family members with the hereditary syndrome. Testing performed in an elaborate laboratory setting would not be as useful for future clinical evaluations, since their specific circumstances could not be approached in most clinics called upon to evaluate the patients. The apparatus and technique used by the author is generally available in almost all otolaryngology out-patient clinics, speech and hearing centers, and in many otologists offices, and the audiograms obtained can be compared with the "typical" tracings presented in this paper. Field screening results have definite limitations as indicated by Corso (11) and, therefore, the field results on the three kindred studied here are not included. Certainly "field" otolaryngology clinical evaluations are also of limited value.

A number of abnormalities were noted on inspection of the ear drum and the author obtained a number of differences in hearing by fork testing to air and bone which necessitated using bone conduction as a more accurate means of documenting the true level of sensorineural reserve. The author appreciates the limitations inherent in bone conduction but is of the opinion that a combination of controlled air and bone testing gives a more accurate picture of the true threshold of hearing than does either alone. In recording the audiometric findings, the level of masked bone conduction was used as the threshold if fork testing at 256 and 512 showed a negative Rinne. Otherwise the pure tone air conduction level was used as threshold.

Ear drums were evaluated for evidence of retraction, healed perforation, immobility, tympanosclerotic plaques, perforation, fluid, abnormal coloration, increased vascularization or operative scar. A careful search was made for any congenital malformations of the pinna, pre-auricular area, canal or upper neck area. Wax was carefully removed from the canal and care was taken to note any potential collapse of the canal with pressure on the tragus.

A screening slit-lamp exam was also carried out on each patient primarily to identify any lenticular abnormalities, since ocular abnormalities have been included as part of the syndrome since Sohar's report (34). An attempt was made to identify cataracts and lenticonus but no cases with these abnormalities were found. One patient had a coloboma and this was the only structural abnormality noted in the 56 patients. No attempt was made to map fundus changes although the fundus was checked in the majority of patients. Accurate fundoscopic evaluations require mydriatic drugs

and the author did not wish to subject volunteers to any potentially hazardous medications Mydriasis would also have permitted more exacting slit lamp exam but the evaluation performed was considered to be critical enough to rule out significant abnormalities Refractive errors were checked with a screening wall chart reading but again no accurate recording of myopia or hyperopia was made because of incomplete eye preparation There were only a few previously diagnosed eye abnormalities in the group and no new abnormalities were identified Tests for dyschromatopsia and intraocular tension were not carried out.

Analysis

The most striking finding in this survey of hearing responses is the paucity of audiometric changes even in those family members manifesting evidence of renal disease Although previous authors have demonstrated distinct sensorineural hearing changes in advanced cases of the hereditary syndrome as mentioned in Part I family members may have very minimal hearing changes and losses attributable to the syndrome may be more apparent than real if care is taken to eliminate other obvious causes of hearing loss particularly those of a conductive nature

Inspection of the groups divided by age and sex reveal a consistent drop at 6 000 cps in both the individual analyses and the average values This finding is not significant for all age groups however This change may be the result of some mechanical defect inherent in the testing apparatus the sound room or the application of the pure tone The author evaluated the first possibility by repeatedly testing volunteers with known audiometric patterns who were frequently used to check calibration of the audiometer No defect in the 6 000 cycle tone was identified An attempt was also made to perform some tests with the door of the sound room open Although other frequencies usually in the 500 cycle range were adversely affected there was no change with testing at 6 000 cps Perhaps the length of the ear canal and the impedance qualities of the ear drum may lead to the creation of a standing wave in the ear canal with a cancelling effect at low intensities of sound The author doubts that such a phenomenon could occur so consistently throughout the testing

In a study of 2891 otoscopically normal children ages 5–14 years Jordan & Eagles (109) indicated that both the median and mean sensitivity was the least at 6 000 cps and this poor sensitivity (higher threshold) continued to be noted in 82 otoscopically abnormal children with impaired mobility discoloration and retraction of the pars tensa This common finding of a drop at 6 000 cps in children under 15 years makes the author s recordings of a loss at 6 000 cps of no significance in this age group

According to the tables prepared by Corso on threshold data by age groups and sex the threshold is higher at 6 000 cps in the age groups 26–49 years for adult males and only in the age group 43–49 for females These data were obtained in otoscopically normal individuals age 18–65 years Since all age groups from 15–65 years in the families with the hereditary syndrome exhibited a drop at 6 000 cps, the finding would appear to be of significance for female individuals over 15 years of age except in the small group 43–49 years. The finding appears to be of minimal significance in the great majority of adult males since they exhibit a drop at 6 000 cps normally according to Corso As mentioned in Part I the hearing disorder is usually progressive in adolescence and young adults with findings in childhood usually normal When this fact is correlated with the audiometric-otological survey of 54 family members, then the value

of the audiometer in identifying potential cases of the syndrome becomes apparent.

Controlled audiometry should probably be performed on all females with evidence of renal dysfunction or with a family history of renal disease. An isolated drop at 6 000 cps should alert the examiner to potential existance of the hereditary syndrome. All females being considered as renal transplant donors should have controlled audiometry in conjunction with a careful otological examination. Because of the aggressive nature of the disease in males, virtually all male patients will have overt signs of renal disease and probably hearing loss after the age of 15 years. Audiometry in these patients will have supplemental value primarily. Audiometric screening should probably be done routinely on adolescent male relatives of patients with the hereditary syndrome, however, in order to identify unknown cases of the syndrome.

The normal finding of decreased hearing sensitivity at 6 000 cps when compared to other frequencies in the age group under 15 years by Jordan and Eagles seems to rule against the virtue of audiometric screening in this age group.

Earlier identification and recognition of the syndrome may permit the salvage of some males with renal transplantation before extensive protein loss and toxemia develop. Earlier identification of the hearing pattern *before* ototoxic drugs have been given for presumed or associated nephritis will aid proper diagnosis of the hereditary syndrome also.

Very little is known of the histological changes in the temporal bone except in two documented cases mentioned previously (5, 74). Internists and pediatricians treating renal disease as well as urologists should be alerted to this gap in our scientific knowledge and encouraged to refer children and young adults with any renal dysfunction for serial audiometry at least yearly and more frequently if a rapidly progressive disorder is present. Otolaryngologists should make a personal effort to secure the temporal bones on these patients. Renal biopsy and renal tissue culture should be encouraged also.

A general susceptibility of kidney disorders may indeed be an inherited phenomenon in the vast majority of renal disease patients. The amino-aciduria mentioned under metabolic patterns may represent an enzymatic transport defect in one organ that perhaps exists in another and by histiochemical studies of renal tissue cultures a better identification of biochemical processes in the sensorineural elements of the ear may be possible. Structure and function must be correlated, however, and only with wide acceptance of controlled audiometry can adequate pre-biopsy, pre-culture and pre-autopsy information be obtained.

New family reports of the deafness and nephropathy syndrome are appearing in the medical literature each year as greater acceptance of the concept of hereditary nephritis occurs. Perhaps the tragic course of the disease in the male can be averted if renal transplant or preventive drugs are used. Certain drugs *have recently* been introduced which protect the ear against the ototoxicity of streptomyces-antibiotics (111) and certain methyl-donor containing amino acids may protect the kidney against nephrotoxic drugs (110). Early identification of the syndrome may permit trial of these substances in hereditary nephritis where a membrane reabsorption defect may exist *similar* to that created by ototoxic drugs.

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The syndrome of hereditary deafness with nephropathy is reviewed and detailed audiometric data in 54 family members of three separate kindred is presented. Controlled au-

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Summary

The syndrome of hereditary deafness with nephropathy is reviewed and detailed audiometric data in 54 family members of three separate kindred is presented. Controlled au-

diometry is recommended for all females with renal dysfunction or a family history of renal disease Young male relatives of affected females should be evaluated periodically for evidence of renal or ear disease and consideration given to using newer preventive and treatment procedures

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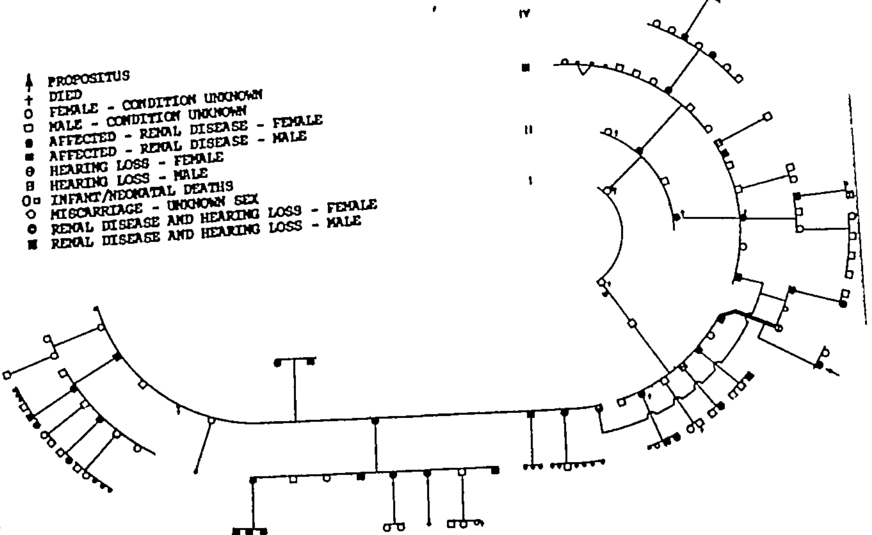


Chart I H, kindred with hereditary deafness and nephropathy

IV

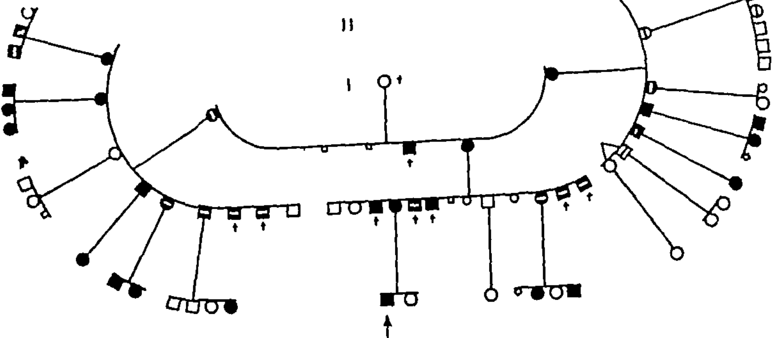
III

II

I

PROPOSITUS
DIED
FEMALE - CONDITION UNKNOWN
MALE - CONDITION UNKNOWN
AFFECTED - RENAL DISEASE - FEMALE
AFFECTED - RENAL DISEASE - MALE
HEARING LOSS - FEMALE
HEARING LOSS - MALE
INFANT/NEONATAL DEATHS
MISCARRIAGE - UNKNOWN SEX
RENAL DISEASE AND HEARING LOSS - FEMALE
RENAL DISEASE AND HEARING LOSS - MALE

Chart II C Kindred with hereditary deafness and nephropathy


PROPOSITUS
DIED
FEMALE CONDITION UNKNOWN
MALE - CONDITION UNKNOWN
AFFECTED - RENAL DISEASE - FEMALE
AFFECTED RENAL DISEASE - MALE
HEARING LOSS - FEMALE
HEARING LOSS - MALE
INFANT/NEONATAL DEATHS
MISCARRIAGE UNKNOWN SEX
RENAL DISEASE AND HEARING LOSS - FEMALE
RENAL DISEASE AND HEARING LOSS MALE
V
IV
III
II
I

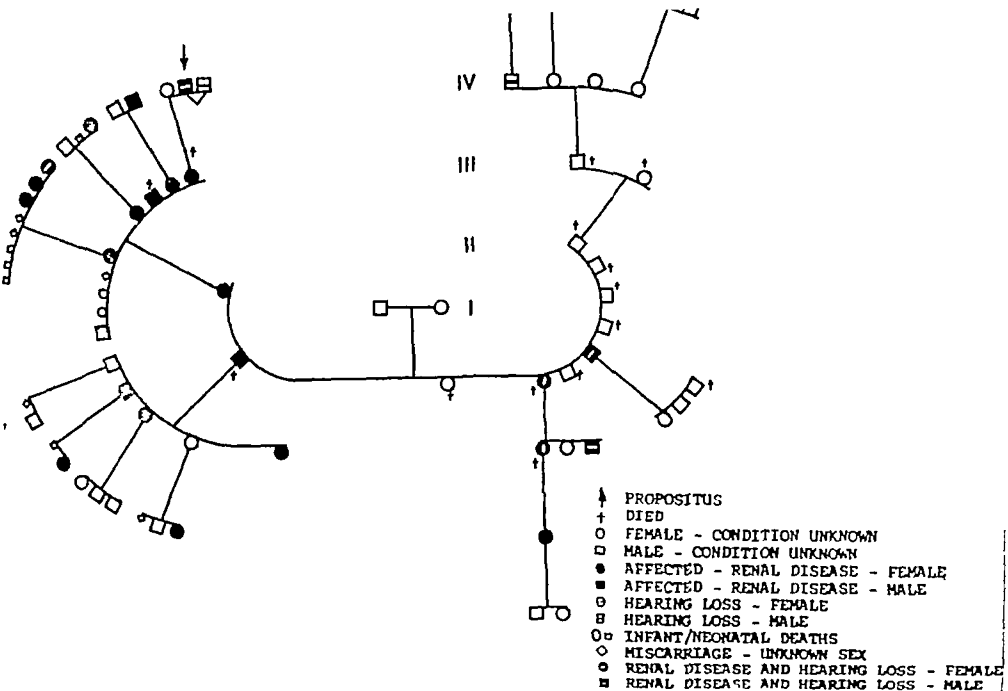


Chart II C Kindred with hered tary deafness and neph opa hy

Chart III M, Kindred with hereditary deafness and nephropathy

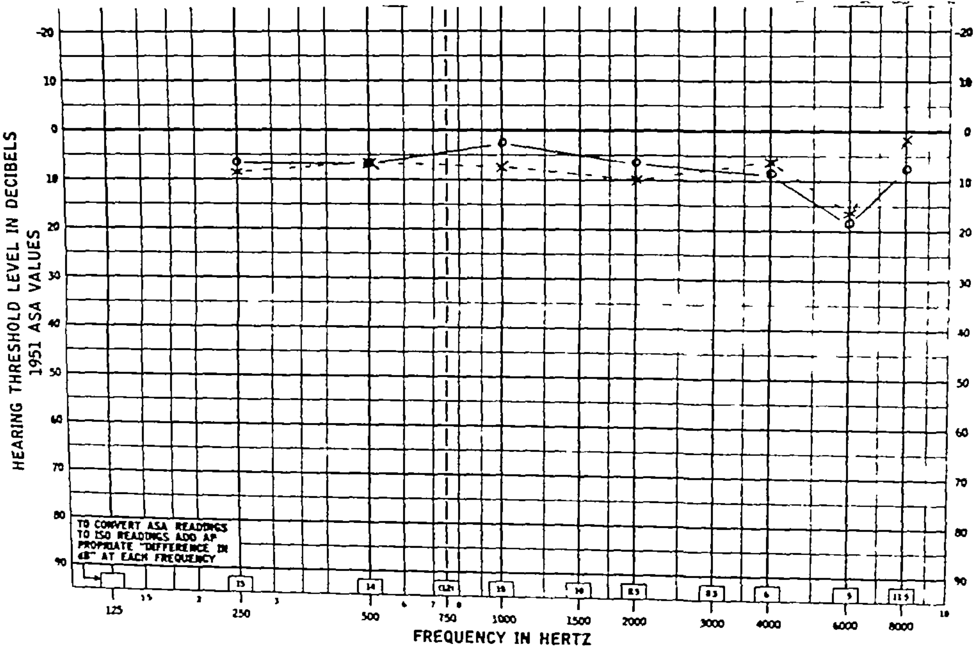


Chart IV Typical udiogram. Age 5–15 male.

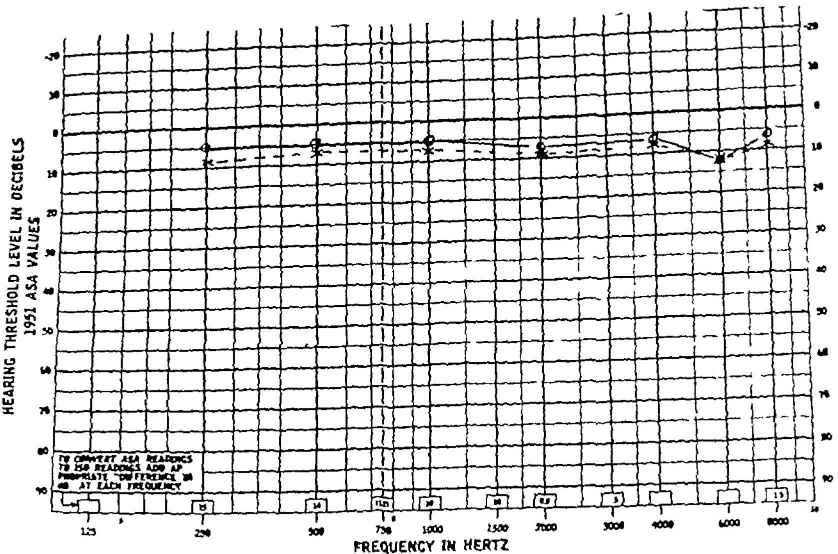


Chart V Typical audiogram, Age 5-15 female.

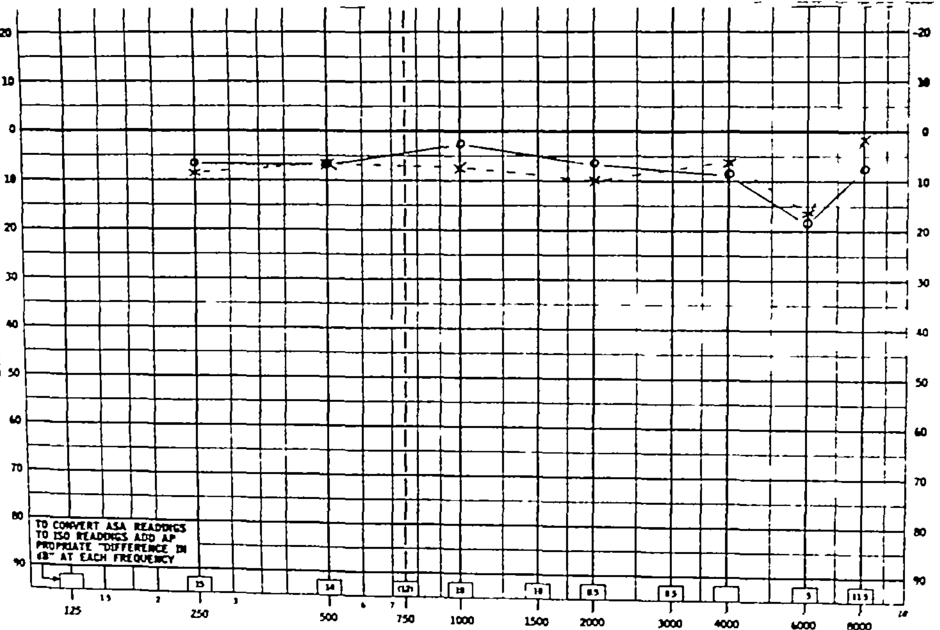


Chart IV Typical audiogram. Age 5–15 male.

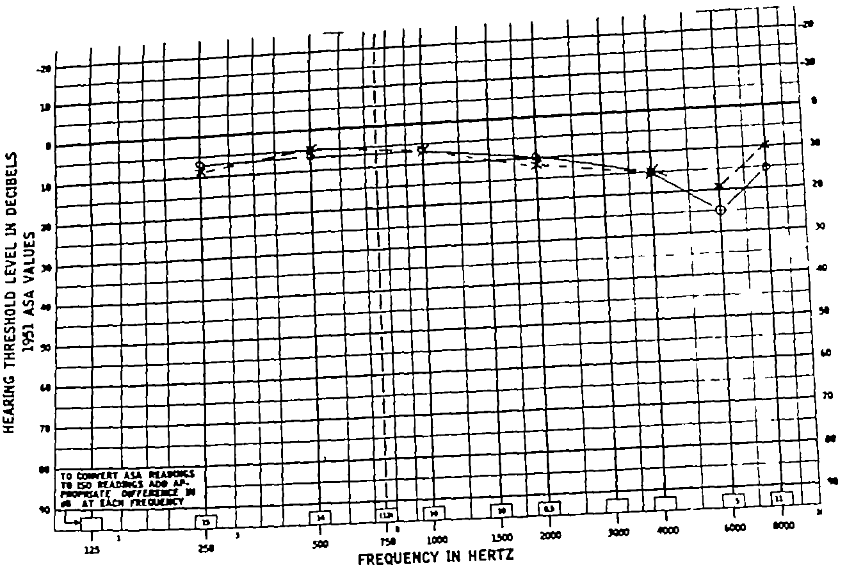


Chart VII Typical audiogram Age 16–49 female.

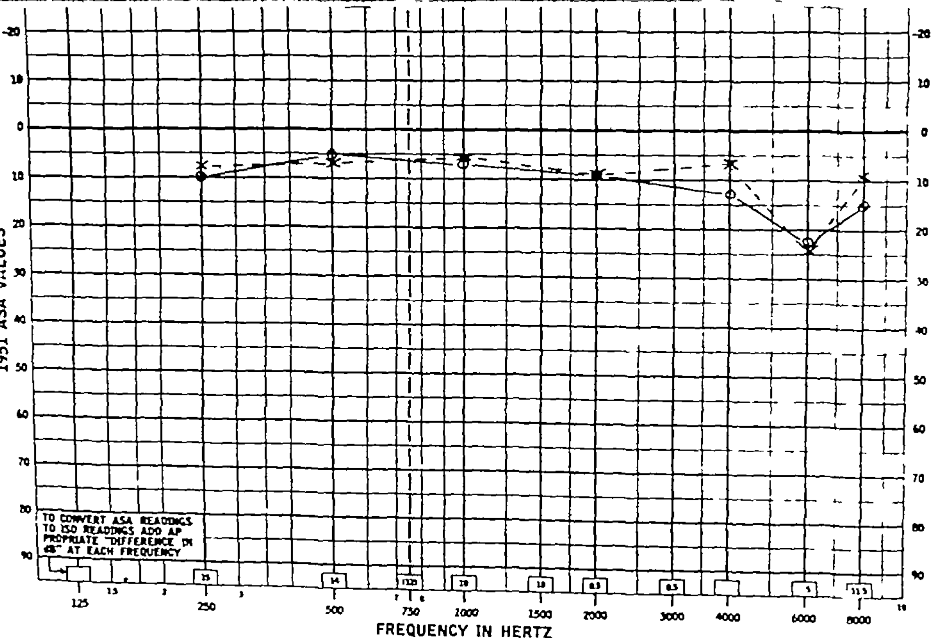


Chart 1.1 Typical audiogram. Age 16–49 male.

Acta

OTO LARYNGOLOGICA

SUPPLEMENT 273

Objective Conditioned-reflex Audiometry in Children

BY

G MOTTA, G M. FACCHINI and E. D AURIA

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CONTENTS

Foreword

In current practice the ear specialist often finds it necessary to establish whether or not a child's hearing capacity is handicapped.

Generally such examination is requested by children's parents or teachers who have noticed a possible retarded phonation or more-or-less pronounced speech disorders. At times the symptomatology of these small patients may appear more complex since as well as phonation disturbances they display an impairment of psycho-intellectual capacity or disorders in locomotor-apparatus functioning, or deficiencies in other sensorial apparata as well as hearing, or yet an association of several of these disorders. Clearly in such conditions audiometric research can become particularly complicated, and the audiologist must be aided in his investigation by the psychologist, neurologist, pediatrician, by a whole team of co-workers, enabling him to give the clinical case a precise framework.

Our work aims at contributing to knowledge of the audiometric techniques based on the study of conditioned reflexes.

In our view these methods of investigation are of considerable interest, since (a) they often achieve particularly satisfying results, as data in the literature show (b) they involve using relatively simple and thus inexpensive equipment and (c) they enable the hearing capacity of patients to be ascertained in the course of consultancy investigation, that is as outpatients without need for admittance or for administering hypnotics or anaesthetics.

In view of this we thought it expedient to make a comparative study as to the usefulness of the various methods of audiometric investigation based on conditioned reflexes, put forward by previous researchers, with a view to establishing the advantages each has over the others (also in relation to the age of examined subjects and their neurological and psycho-intellectual as well as hearing handicaps) and acquiring more thorough knowledge of them and possibly making them easier to implement.

In our survey we took into account 316 subjects whose ages varied from 1 to 5 years.

It is to be pointed out first of all that our cases always displayed such behavioural reactions as to lead to suspicion of a possible hearing impairment, thereby warranting audiometric examination.

Specifically these were subjects displaying the following isolated or variously associated disorders.

(i) More-or-less severe hearing deficiency
(ii) Retardation or rather alterations to varying extent in speech.
(iii) Neurological disturbances associated with low I.Q.

In view of the clinical characteristics of the patients examined, our statistical data can be compared with those of Authors who have only studied normal individuals with proper caution, allowing for the greater difficulties we ourselves encountered.

Our cases were subjected to audiometric investigation, and based upon study either of (a) psychogalvanic reflex, or (b) conditioned instrumental reflexes, or (c) conditioned orientation reflexes.

All patients were examined by at least two of these techniques, with a view to confirming and integrating the data obtained with each method of investigation.

In a separate appendix to this work we further study and discuss the validity of the

above audiometric techniques for determining the hearing capacity of patients who are particularly complex from a clinical viewpoint

These were cases where, owing to the simultaneous presence of lesions to various sensorial apparata or the existence of severe neurological or psycho-intellectual handicaps, the audiometric examination presented unquestionable difficulties

The data on these cases, though not sufficiently numerous to permit statistical considerations, nevertheless contribute to knowledge of the possibilities and limitations of the audiometric techniques taken into account by us.

We must point out, lastly that all the subjects were studied during consultancy-type investigations, and thus without admitting them. In some more complicated cases, however it was necessary to repeat and complete the examination in subsequent sessions, at an interval of hours or even days between each. In doing this we always allowed for the fact that in infant audiometric investigations it is advisable to avoid tiring or irritating the subject if one wants his full co-operation, and hence more accurate data.

CHAPTER 1

Psychogalvanic or Electrodermal Reflex

1 PREVIOUS RESEARCHES

(a) Physiological premises

In 1888 Féré first discovered that skin resistance to the passage of electric current underwent changes as a result of emotive phenomena or sensorial excitation of varied nature These changes could be measured accurately by a milliampermeter Féré called the phenomenon Psychogalvanic Reflex (P G.R.).

Several theories have been put forward by different Authors to explain this typical reflex reaction: lowered skin resistance as a result of an emotive stimulus of varying nature has been related to changes in sweat-gland secretion (Darrow 1934) as well as to variations in local circulation (Goadby & Goadby) But the possibility that reactions of the muscular fibres interfere with the mechanisms whereby P G R. is governed has been ruled out (Duret-Cosyns & Duret)

The changes in sweat-gland secretion and consequent variations in skin *electrical* resistance, which arise as a result of emotive phenomena of varying nature are unquestionably secondary to neurovegetative reflexes by stimuli which according to the majority of Authors, run mainly in the sympathetic fibres. Wells & Forbes have however uphold possibility that a certain number of these stimuli also follow the parasympathetic fibres.

Various investigations have been carried out to establish what the physiological phenomena are that govern P G.R., and principally what nerve centres condition and regulate its appearance

According to some researchers (Wang & Lu, 1930 Wang & Mok Darrow 1934 Spiegel & Hunsicher Swartz) this reflex depends on the activity of the cerebral cortex and, in particular on that of the premotor area. Others (Kennard Craig & Hare) would have it that they are under the control of subcortical and specifically thalamic centres.

The first hypothesis seems to find wider consensus.

In this connection Duret-Cosyns & Duret conducted investigations demonstrating that central perception of the stimulus by the subject is an essential element for the onset of P G R. sleep, narcosis, coma and interruption of the *sensitive fibres of the* stimulated area in fact nullify any response whatever characteristic the stimulus may have

Studies on P.G.R. have taken on particular importance especially from the clinical viewpoint, since Bordley Hardy & Richter in 1948 thought of using this reflex to establish the hearing capacity of a subject not referring to his own responses (subjective audiometry) but to objective data directly detectable by the examiner and uninfluenced by the subject s greater or lesser degree of co-operation. These researchers first of all found that a sudden auditory stimulus with intensity of at least 40 dB above threshold could in fact cause the onset of P G R. But this reflex is scarcely utilizable for practical purposes indeed, besides having a particularly high intensity the auditory stimulus must reach the subject suddenly so as to provoke an emotive reaction of some entity Moreover this reaction exhausts easily when

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such tender age to understand the mechanism of play audiometry

Conversely Borel-Maisonny observed differences at times more than 40–50 dB for the various frequencies in children aged between 3 and 6

Pellegrini revealed threshold differences of over 30 dB in children under 3 years of age, while these fluctuated between 5 and 20 dB in adults

Pesavento, on the basis of a case-list of 56 children with ages varying from 2 to 5 years, was able to establish the following:

(*a*) P G R. study achieves threshold values generally corresponding with considerable approximation to those obtained by using the Peep-show (cf instrumental conditioned reflexes—Page 17)

(*b*) In children aged between 3 and 5 the threshold values found by P G R. study can be about 10 dB above the real

(*c*) In children between 2 and 3 years of age audiometry by way of P G R. study is sometimes the only practicable investigation method even though it often gives threshold values higher than the real—the error may be as much as 20–25 dB above real threshold.

Sortini studied 250 preschool-age children and reported having satisfactorily assessed the hearing in all cases through P G R. examination He was able to discuss the possibility of phonetic training in 174 cases on the basis of the data so obtained

Germanà sought the hearing threshold for 6 frequencies (250, 500 1 000, 2 000 3 000 and 4 000 c s) in 20 children aged between 6 and 13 attending a School for the Deaf in order to highlight any residue exploitable for speech-auditory training He made a comparative study of the audiometric curves drawn from subjective tone audiometry and P G.R. study In the latter the threshold was higher in every case the difference in curves obtained by the two methods varied *from 5 to 25 dB*, and was always more sensitive in *younger subjects*, especially in the acute-frequency range.

Barr who investigated 20 adults, found no significant difference between threshold values obtained through P G.R. study and by tone audiometry

Dupon-Tersen affirmed the importance of audiometry through P G.R. study in the case of 2 and 3-year-old children. He pointed out, however that this method can give rise to errors in these subjects, since a threshold 30–40 dB higher than the real is always obtained Consequently judgment as to the possibilities of training them cannot be based solely upon data drawn from this examination.

Lüscher & Kusen researched on three groups of subjects whose ages ranged from 6 to 60 and detected very slight differences between threshold values obtained through P G.R. study and from using the peep-show or classical tone audiometry The threshold by the former method was in fact always higher than the real. The difference fluctuated between 0 and 20 dB in the control group of 15 normal-hearing subjects, 0 and 10 dB in the group of 26 hard-of hearing subjects aged between 6 and 60 and in the one comprising 23 deaf-mutes with ages ranging from 7 to 16 In some cases these differences could however be higher reaching 30 dB The Authors conclude that the reliability of audiometric values obtained through P G.R. study is considerably greater in impaired-hearing than in normal-hearing subjects.

Grisanti *(1958) studied* the ratio between hearing threshold obtained through simple, i.e., unconditioned, P.G.R. study and by subjective tone audiometry He detected an average difference fluctuating between 20 and 45 dB But this deviation would be appreciably increased in correspondence with the acute frequencies. Grisanti related this phenomenon to the lower emotive capacity of these frequencies

Baron & Dejour subjected to control by classical tone audiometry 25 children previously examined by P G R. over a period of 10 years. They reported that in the majority of children —aged between 1 ½ and 4 at the time of first examination—the control substantially confirmed, with a threshold difference varying from 10 to 15 *dB*, the audiometric curves defined

the examination is prolonged, and it is hardly possible to define the subject's audiogram under such conditions Bordley et al then thought of conditioning P G R. on the principles substantiated by Pavlov's observations More specifically they proceeded as follows routing an auditory stimulus of surely supraliminal intensity and after a few seconds a slightly painful electric shock The latter brought about the onset of obvious P G.R. These stimulations were repeated a certain number of times It was observed at a given moment that the sound stimulus caused P G R. to make its appearance even though it was not followed by the pain stimulus At this moment the patient could be regarded as conditioned any sound stimulus, even of intensity slightly above threshold, could bring about the appearance of P G R.

(*b*) Determining the hearing threshold through study of psychogalvanic reflex

The Bordley Hardy & Richter experiments were later taken up by numerous Authors who used conditioned P G R in studying the hearing capacity under different clinical and experimental conditions, and in particular for determining the hearing threshold in children. These researches could be conducted by using appropriately designed devices (psychogalvanometers) These afford a graphic recording where in the stimuli routed to the subject and changes in his skin resistance due to the psychogalvanic reflex are given

Bordley Hardy & Richter as well as Authors who later dealt with the subject first attempted *to establish whether the hearing threshold determined* through P G R study tallied approximately *enough with the* one detected by classical tone audiometry

It is to be noted in this regard that the results of researches conducted by these Authors on adults or school age children are fairly in agreement and demonstrate that objective audiometry based on P G R. study achieves sufficiently reliable data on the hearing threshold

Bordley & Hardy studied 700 subjects in this connection They achieved positive results in *95% of examined cases More specifically* they observed an average difference between the *hearing threshold* by *classical tone audiometry* and the one obtained through P G R. study of 2.46 dB in adults and 3 60 *dB in children aged* between 4 and 14

Maspétiol Gourgerot & *Konne revealed that* the threshold obtained through P G.R. study was higher (and thus the hearing loss more severe) than that drawn from conventional tone audiometry by 10–20 dB in adults and children over 8 years of age This difference rose to 15–25 dB in subjects aged between 4 and 8 to 20–30 dB in 3–4-year-olds and 30–40 dB in 2–3 year-olds These Authors also pointed out the frequency of failures in subjects under 3 years of age

Conversely Faure, Portmann, Dutertre & Bramerie observed that the threshold differences in adults were contained to within 2 and 3 dB but they rose to 10 dB in subjects aged between 4 and 7 to 15–20 dB in 2 1/2- to 4-year-olds and to 20–30 dB in those under 2 1/2.

According to Pagano who studied children aged between 4 and 7 the difference between the threshold obtained by examinations via P G R study and the one detected by classical tone audiometry does not exceed 10–15 dB

Doeffler & McClure examined 30 adult subjects and detected *an average difference* of 3 50 dB between the threshold by traditional tone *audiometry and the one* obtained through P G R. study

Barr performed examinations on 324 children aged between 1 and 6 In subjects over 2 1/2 years of age he obtained in 85% of cases threshold values with P G R study that agreed with the ones detected through Play Audiometry (cf instrumental conditioned reflex audiometry—Page 17) In children less than 2 1/2 years old he was able with P G R study to gather significant data in only 50% of examined cases, but nevertheless *upheld the validity of* the method for this group too in view of the *frequent impossibility of getting children of*

such tender age to understand the mechanism of play audiometry

Conversely Borel-Maisonny observed differences at times more than 40–50 dB for the various frequencies in children aged between 3 and 6

Pellegrini revealed threshold differences of over 30 dB in children under 3 years of age, while these fluctuated between 5 and 20 dB in adults.

Pesavento on the basis of a case-list of 56 children with ages varying from 2 to 5 years, was able to establish the following:

(a) P.G.R. study achieves threshold values generally corresponding with considerable approximation to those obtained by using the Peep-show (cf instrumental conditioned reflexes—Page 17)

(b) In children aged between 3 and 5 the threshold values found by P.G.R. study can be about 10 dB above the real

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especially if the examination is repeated after an interval of some days and its practical value is controlled at a distance of 5 to 6 years. Indeed, they hold that P G R study is the only method that allows immediate fitting of a hearing aid and adequate training.

Bedoni examined through P G.R. 10 subjects affected by a more-or-less high degree of post traumatic perceptive deafness, in order to seek what he calls the Soglia di Incomoda Audibilità (S.I.A. = Uncomfortable Audibility Threshold) i.e. the lowest sound intensity capable of bringing about the onset of P G.R. According to Bedoni, this investigation procedure has given positive results also for medicolegal purposes it would be capable of providing useful elements for localizing the auricular lesions responsible for post-traumatic hearing deficiencies.

Grisanti (1967) carefully investigated by spontaneous—i.e. unconditioned—P.G.R. 128 children under 10 years of age, and 72 adult patients. He reports that it was possible to complete the examination in the child group, obtaining enough indication as to subjects auditory conditions in the majority of cases (81.5) But the examination was not crowned with success in the remaining 18.5 owing either to absence of a psychogalvanic reaction (9.8) or to the small patient's behaviour (8.7).

Bearing in mind the patients ages, Grisanti maintains that failures generally occur in very small children for the following reasons.

1 Within the first and second year of life psychogalvanic reactivity may be lacking or be very slight due either to a lower activity of the sympathetic system, or to high skin impedance

In 4- and 5-year-old children, spontaneous R G R study (without recourse to faradic shock) can also give rise to reactions, and to agitation and crying in particular

In the adult group simulated deafness was successfully ascertained in 52.3 % of cases, psychogenic deafness in 10.8 and real deafness in 35.3 %.

Grisanti therefore maintains that audiometry based on P.G.R. study besides being the only practical method of assessing hearing in children under 4 years of age, is a valid aid to diagnosing simulated or non-organic deafness.

In a later research (1968) Grisanti subjected a group of 18 normal-hearing individuals of different ages to voice audiometric investigations through P G.R. study Complete-sense disyllables were routed into the earphones and intercalated by the word "scossa" (shock) which was combined with a faradic shock.

The combination was repeated until a constant psychogalvanic reflex was obtained. Once conditioning was established the intensity of the phonemes was reduced until the words proved no longer to be intelligible though still heard by the patient: clearly the psychogalvanic reflex did not come about in such conditions.

Grisanti maintains that the procedure he used may prove very useful in studying auditory lesions of central origin and in simulators who complain of more marked deafness for speech than for pure tones.

Piragine & Bazzana found that when performing audiometry through P G.R. study difficulties may be encountered in relation to the variations presented by sweating in the different subjects under examination. They therefore studied the changes brought about in this reflex by two parasympathotropic drugs: pilocarpine which, as is known, induces sweat, and atropine which inhibits it. The investigation was conducted on 9 subjects chosen for their ready conditionability and on 4 patients who were hard to condition. On the basis of their results the Authors report that pilocarpine promotes the onset and increases the amplitude of the responses in relation to P G R., while atropine produces an opposite effect. These data can be utilized in current practice: it is in fact to be borne in mind among other things that some particularly expert simulators might make use of atropine to mask the results of audiometric tests based on P.G.R. study

Despite the lively interest aroused in many researchers by objective audiometry based on

4–5 years earlier through P G R study They thus maintain the usefulness of this investigation method for diagnosing deafness in children where subjective" audiometry is impracticable *owing to their age. Baron & Dejour also* affirm the need to compare the results from *P G R. study with those obtained by free field* voice audiometry and if possible supplement *them by other medico-educational and E.E.G* examinations, especially when disturbances of *the central nervous system are suspected.*

Fedele recently studied 32 children aged between *2 and 8* and whose mental age was noticeably lower A group of 16 with ages ranging from 2 to 5 were examined through P G R. and peep-show Fedele reports having on the whole achieved satisfactory results in these cases with both investigation methods He further affirms that a comparative study of data drawn from the two methods demonstrates audio-psychogalvanometry often to be a useful complement to investigation with the peep-show even though the threshold by P G R is usually somewhat higher generally over 10–15 dB in fact.

To conclude, therefore the investigations by different Authors into the possibilities of conditioned P G R. in audiometry do not always furnish perfectly consistent data

The researchers who examined adults usually maintain that the hearing threshold obtained through P G R study tallies substantially with the one by classical tone audiometry (Bordley & Hardy 1949 Faure Portmann Dutertre & Bramerie, 1952 Doerfler & McClure 1954 Burk 1958) Nevertheless, in these subjects too some Authors observed differences flutuating between 5 and 20 dB in the thresholds defined by the two methods (Maspétiol Cougerot & Korine 1951 Pellegrini, 1955) These differences are generally very much more noticeable in children and become more marked as the age lowers. According *to some (e g., Pesavento, 1957)* the threshold differences for children under *3 vary from* 20 to 25 dB and to others (Maspétiol Gougerot & Korine, 1951 *Dupon-Tersen 1958) from* 30 to 40 dB

(c) Possibilities of using psychogalvanic reflex in infant audiometry

The usefulness of audiometric investigations based on P G R. study has been maintained by several researchers, not only because this method enables the hearing threshold to be defined even without the patient s active co-operation but because it reveals simulated deafness and sometimes identifies within certain limits the hearing losses due to lesion of the central nervous system

Faure Portmann & Portmann examined subjects *affected by various types of deafness.* They reached the conclusion that the objective audiogram obtained through P G R study enables the extent of hearing deficiency due to lesions of the peripheral structures of the hearing organ to be established with sufficient accuracy Much more complicated however is interpretation of the data found by this method in cases of hearing loss through lesion of the nerve centres wherein according to these Authors it is often useful also to perform an E.E.G examination

Manfredi (1952) affirmed the importance of P G R. study in audiometry He later (1954 1956) brought modifications to the classical method conditioning the subject with a stimulus induced by a voltaic rather than galvanic current (psychovoltaic reflex) He reports having been able by this method to perform the examination on patients from whom due to the small amplitude of reflexes, it had not been possible to achieve reliable results by the ordinary process.

Goldstein (1956) studied 20 subjects displaying psychogenic deafness. In 10 cases he *failed to achieve conditioning,* while in the other 10 unreliable graphs were recorded although the *conditioning* was obtained Goldstein thus considered it advisable to perform an electroencephalogram in addition to the P G R study in these cases

Baron Roul & Thalamot affirm that investigations through P G R study are a valid aid to early diagnosis *of deafness in children,*

Initially studied were 24 subjects 6 to 8 years old, taken at random in a school for mentally handicapped children. This group had however to be re-set up because the percentage of failures was too high. The Authors therefore asked teachers to point out the individuals most likely to co-operate. Thus 34 subjects whose average I.Q. was 0.37 were selected. In only 24 of these cases, however could reliable recordings be obtained.

We think it important in this connection to draw attention to a datum given by these Authors: of the 24 children for which a full recording was made, 13 were mongoloid, and hence very tranquil subjects. In the 10 children where an audiogram could not be obtained, the causes of failure were: negativism, hyperactivity shyness total rejection and, in one case, even a violent cough at the start of the test.

With regard specifically to the examinations performed by conditioning the patients with a light stimulus it is to be pointed out that some had fear reactions while others even went to sleep during the test.

On the whole the Authors concluded that only in a small percentage of mentally deficient children can reliable P.G.R. recordings be secured. They therefore questioned, in accord with Irwin, Hind & Aronson, whether audiometric research by such a painstaking method a psychogalvanic examination is justified when there are other techniques easier to perform. They pointed out in this connection that it is difficult to execute a reliable audiogram by P.G.R. study for a subject with mental handicaps who is incapable of co-operating in the investigation conducted by ordinary infant audiometry techniques.

More recently a careful analysis of the possibilities of P.G.R. study within the infant-audiometry field was done by Préfot & Lafon, who found that failures can occur with normal-hearing children by this technique either due to high skin impedance or to impossibility of provoking an emotive shock in the child owing to its age or lastly to the impossibility of achieving conditioning even after repeated tests.

Préfot & Lafon also noted that in the course of P.G.R. study into severely deaf children, interferences related to accidental stimuli overlapping the sound stimulus can be recorded in the tracings and these of course affect the results of the examination. They therefore conclude that audiometry through P.G.R. study has not fulfilled the hopes raised by the first experiments of Bordley and colleagues. In current practice this technique is delicate and offers numerous possibilities of error especially in the case of subjects of tender age. It tends for these reasons to be abandoned as an investigation method for assessing the hearing capacity of preschool age children.

2. PERSONAL RESEARCHES

(a) Investigation method

For the study of P.G.R. we adhered to the examination procedure adopted consistently by the majority of Authors who have dealt with the subject. More specifically we used a Portmann-Racia psychogalvanometer (*Fig. 1 A*) and a Grason-Stadler psychogalvanometer (Fig. 1 *B*) both equipped with two pens, one tracing the tone and possibly pain-stimulation routed to the subject, and the other the changes in skin resistance.

The examinations were performed in a perfectly silent cabin. The child was put on his mother's or the assistant's knee and was kept amused by different toys or illustrated books (*Figs. 1 A, 1 B* and *2*).

We applied two electrodes to the sole of his foot, to detect the variations in skin resistance and the ones transmitting the faradic stimulation to the opposite calf (Fig. 2). After calibrating the device the examination was started.

The examination was initially conducted on each subject in "free field" i.e., routing the sounds to a speaker located at a suitable distance from him. Later when satisfactory conditioning was established, the hearing threshold of both ears was analysed, routing the sound stimulus into the relative earphones.

P G R. study it has not always given satisfactory results in practice and has therefore been the object of criticism, especially with regard to its use in children who are very small or of low I Q., or at any rate with lesions of the central nervous system

Goldstein Polito-Castro & Daniels studied 32 normal hearing children aged between 7 and 13 They report 14 of them to have been scarcely conditionable by P G R

Statten & Wishart examined 122 children between 1 ¹/₂ and 6 years old Only in 15% of these cases did they succeed in plotting a *satisfactory audiogram. In 70% the tracing* obtained was only indicative According to these Authors *the possibilities of using the method* are limited, either because conditioning of the subjects under examination *is not established* and the skin resistance changes are slight in response to the auditory stimuli, or because there are psychological reactions on the part of the small patients that prevent completing the investigation

On the other hand since the majority of children can be examined by procedures more agreeable to them (conditioned instrumental or orientation reflex audiometry) according to these Authors, P G R. should be attempted only in cases where such types of investigation are impracticable owing, for example to severe motor deficiencies in the limbs or cephalic trunk, or to specific psychological reactions.

Celis-Perez subjected to audiometric investigation with P G R. study 25 children in early infancy 112 at pre-school age and 10 adults He found it impossible to achieve conditioning in some cases and in others there was not even response to the faradic stimulus On the other hand he also noted that a sedative premedication used in certain subjects to lessen their distress, generally causes a delay in the onset of reflexes With this in mind Celis-Perez holds that the results obtained with P G R. study are only reliable when *at least two equal audiograms* have been had in the course of tests carried *out at a distance* of some days between each

Slanger & Gottsleben, *after* conducting a set of experiments through P G.R. study on low I Q mentally handicapped *subjects, maintained* that this technique does not often allow reliable results to be obtained in such patients

Irwin, Hind & Aronson who investigated 20 mentally handicapped subjects aged between 9 and 38 with an average I Q of 0.50 found audiometry based on P G R study to give less satisfactory results than does conventional audiometry About one quarter of their case-list could not be examined by P G R., either because of their particular reactions or due to *difficulty in establishing conditioning.*

Schultz, starting from the assumption that *failures* in audiometry based on P G R. are often due to the fact that faradic shock provokes *a painful or at any rate unpleasant sensation* in small patients, replaced this stimulus with a luminous one (a Nitra 500 Watt photolamp) producing a sudden flash in a dark room The conditioned reflex was obtained by repeatedly combining the sound stimulus with the luminous More specifically Schultz first routed the pure tone and, after two or three seconds, the light stimulus which lasted about one second. By this method he studied 29 hearing-defective children aged between 1 and 7 and reports the examination to have been unsuccessful in 4 children, while the hearing capacity of one ear could be *determined in 16 and of both* ears in 9

Copelmann basing on results of a series of researches conducted via P G R discusses the importance of emotional factors in the nervous mechanisms that govern auditory perception

Kodman, Fein & Mixon obtained useful information in 28 of the 31 mentally handicapped children they examined by way of P G R

Moss Moss & Tizard carried out careful investigations to establish the results that are obtainable through *P G R. study in mentally* handicapped children. The patients were generally *subjected to* two tests one using electric shock as a conditioning stimulus, and the other an intense light (as Schultz had previously propounded)

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Kodman Fein & Mixon obtained useful information in 28 of the 31 mentally handicapped children they examined by way of P G R

Moss, Moss & Tizard carried out careful investigations to establish the results that are obtainable through P G R. study in mentally handicapped children The patients were generally subjected to two tests one using electric shock as a conditioning stimulus, and the other an intense light (as Schultz had previously propounded)

Initially studied were 24 subjects 6 to 8 years old, taken at random in a school for mentally handicapped children. This group had however to be re-set up because the percentage of failures was too high. The Authors therefore asked teachers to point out the individuals most likely to co-operate. Thus 34 subjects whose average I.Q. was 0.37 were selected. In only 24 of these cases, however, could reliable recordings be obtained.

We think it important in this connection to draw attention to a datum given by these Authors: of the 24 children for which a full recording was made 13 were mongoloid, and hence very tranquil subjects. In the 10 children where an audiogram could not be obtained, the causes of failure were: negativism, hyperactivity, shyness, total rejection and, in one case, even a violent cough at the start of the test.

With regard specifically to the examinations performed by conditioning the patients with a light stimulus, it is to be pointed out that some had fear reactions while others even went to sleep during the test.

On the whole the Authors concluded that only in a small percentage of mentally deficient children can reliable P.G.R. recordings be secured. They therefore questioned, in accord with Irwin, Hind & Aronson, whether audiometric research by such a painstaking method as psychogalvanic examination is justified when there are other techniques easier to perform. They pointed out in this connection that it is difficult to execute a reliable audiogram by P.G.R. study for a subject with mental handicaps who is incapable of co-operating in the investigation conducted by ordinary infant audiometry techniques.

More recently a careful analysis of the possibilities of P.G.R. study within the infant audiometry field was done by Prélot & Lafon, who found that failures can occur with normal-hearing children by this technique either due to high skin impedance or to impossibility of provoking an emotive shock in the child owing to its age, or lastly to the impossibility of achieving conditioning even after repeated tests.

Prélot & Lafon also noted that in the course of P.G.R. study into severely deaf children, interferences related to accidental stimuli overlapping the sound stimulus can be recorded in the tracings and these of course affect the results of the examination. They therefore conclude that audiometry through P.G.R. study has not fulfilled the hopes raised by the first experiments of Bordley and colleagues. In current practice this technique is delicate and offers numerous possibilities of error especially in the case of subjects of tender age. It tends for these reasons to be abandoned as an investigation method for assessing the hearing capacity of preschool-age children.

2. PERSONAL RESEARCHES

(a) Investigation method

For the study of P.G.R. we adhered to the examination procedure adopted consistently by the majority of Authors who have dealt with the subject. More specifically we used a Portmann-Racm psychogalvanometer (*Fig. 1 A*) and a Grason-Stadler psychogalvanometer (Fig. 1 *B*), both equipped with two pens one tracing the tone and possibly pain-stimulation routed to the subject, and the other the changes in skin resistance.

The examinations were performed in a perfectly silent cabin. The child was put on his mother's or the assistant's knee, and was kept amused by different toys or illustrated books (Figs. 1 *A*, 1 *B* and 2).

We applied two electrodes to the sole of his foot, to detect the variations in skin resistance, and the ones transmitting the faradic stimulation to the opposite calf (Fig. 2). After calibrating the device the examination was started.

The examination was initially conducted on each subject in "free field", i.e., routing the sounds to a speaker located at a suitable distance from him. Later when satisfactory conditioning was established, the hearing threshold of both ears was analysed, routing the sound stimulus into the relative earphones.

P G R study it has not always given satisfactory results in practice and has therefore been the object of criticism especially with regard to its use in children who are very small or of low I Q or at any rate with lesions of the central nervous system

Goldstein Polito-Castro & Daniels studied 32 normal-hearing children aged between 7 and 13 They report 14 of them to have been scarcely conditionable by P G R

Statten & Wishart examined 122 children between 1 $^1/_2$ and 6 years old Only in 15% of these cases did they succeed in plotting a satisfactory audiogram In 70% the tracing obtained was only indicative According to these Authors, the possibilities of using the method are limited either because conditioning of the subjects under examination is not established and the skin resistance changes are slight in response to the auditory stimuli or because there are psychological reactions on the part of the small patients that prevent completing the investigation

On the other hand, since the majority of children can be examined by procedures more agreeable to them (conditioned instrumental or orientation reflex audiometry) according to these Authors P G.R should be attempted only in cases where such types of investigation are impracticable owing, for example, to severe motor deficiencies in the limbs or cephalic trunk or to specific psychological reactions

Celis-Perez subjected to audiometric investigation with P G R. study 25 children in early infancy 112 at pre school age, and 10 adults. He found it impossible to achieve conditioning in some cases, and in others there was not even response to the faradic stimulus On the other hand he also noted that a sedative premedication used in certain subjects to lessen their distress, generally causes a delay in the onset of reflexes With this in mind Celis-Perez holds that the results obtained with P G R study are only reliable when at least two equal audiograms have been had in the course of tests carried out at a distance of some days between each

Slanger & Gottsleben after conducting a set of experiments through P G.R. study on low I Q mentally handicapped subjects, maintained that this technique does not often allow reliable results to be obtained in such patients.

Irwin Hind & Aronson, who investigated 20 mentally handicapped subjects aged between 9 and 38 with an average I Q of 0.50 found audiometry based on P G R. study to give less satisfactory results than does conventional audiometry About one quarter of their case-list could not be examined by P G R., either because of their particular reactions or due to difficulty in establishing conditioning.

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Fig. 2. Audiometric examination by P.G.R. study. On her mother's lap the child is entertained with a story-book. Note the electrode arrangement.

The chief causes of failure were agitation and subsequent crying, which often followed upon the first faradic shock.

(b) Results

Our data on the 35 patients we examined are given in Table 1.

The results obtained in the different cases were classified into three groups, i.e.

Negative: when there was no precise indication as to the patient's hearing capacity.

Indicative: when it was managed to establish whether the subject was normal-hearing, hard-of-hearing or severely deaf, but without being able to define the hearing threshold for the various examined frequencies.

Positive: when the thresholds for the explored tones could be ascertained especially for 250, 500, 1 000, 2 000 and 4 000 c/s which, as we know, are the frequencies included within conversational voice.

Taking overall the data in Table 1 relative to the 35 patients, it is to be seen that: for 13 (37 %) the examination did not enable useful data to be found; for 18, i.e. about 51 %, only indicative data were derived; for 4 or 11 % only sufficiently accurate threshold values could be achieved.

Table 1. *Results obtained for 35 patients examined through P.G.R. study*

Age (years)	No. cases	Results: Negative		Indicative		Positive	
1–2	8	5	62.5	3	37.5	—	
3	13	4	30.8	7	53.8		15.4
3–4	14	4	28.6	8	57.1	2	14.3
Total	35	13	37.1	18	51.4	4	11.5

A more detailed analysis of these data also reveals that:

1. The failure percentage was very high in subjects under 2 years of age (62 % negative results): only in a somewhat modest proportion (37 %) was it possible to have indicative data.

2. In patients over 2 the percentage of negative results progressively lowered while the number of cases wherein indicative data were obtained increased (about 54 %) but the proportion for which really useful data were obtained (positive results) was always extremely low, i.e., around 15 %.

(c) Considerations

As we have mentioned, the opinions of different researchers into the validity of psychogalvanometry and especially into the expediency of using this technique in consulting-room practice for audiometric investigation into pre-school-age or low I.Q. subjects, are rather discordant.

In our view, audiometric examination based on P.G.R. study presents three basic aspects: one positive and two highly negative.

The positive element in this investigation method, on the theoretical and practical plane, is chiefly pavlovian conditioning, which rules out any active participation in the examination by the small patient.



Fig 1(A–B). (*A*) A 4 year old examined audiometrically by P G.R. study (Portmann-Racia Psychogalvanometer). Letting him have toys helps to establish contact, attenuate his reactions to the painful stimulus and get him interested in the examination. (*B*) Audiometric examination of a 3 year-old by P G R. study (Grason-Stadler Psychogalvanometer marketed by Amplifon)

We began the investigation by routing into the earphone a deep-frequency sound (250 or 500 c/s) whose intensity was definitely supraliminal The task of this stimulus was to test the subject's reactivity The patient was then conditioned by combining the sound stimulus with faradic stimulation, at an interval of a few seconds When conditioning was achieved we routed the sound stimulus only progressively reducing its intensity 10 dB at a time, in order to establish the threshold which tallied with minimum intensity of the stimulus capable of bringing about a skin reaction At intervals, when the psychogalvanic reflex tended to expire it was revived by making a faradic stimulus precede the sound one. The various frequencies were tested by this technique until a complete audiometric curve was found.

To check reliability of the data obtained, the results of the P G R examination were always verified by other types of objective audiometry i.e instrumental conditioned reflex (I C.R.) or conditioned orientation reflex (C.O R.) study



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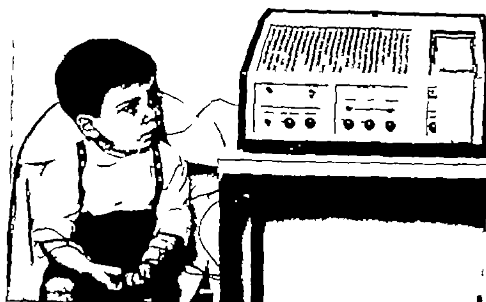


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In our view audiometric examination based on P.G.R. study presents three basic aspects: one positive and two highly negative.

The positive element in this investigation method, on the theoretical and practical plane, is chiefly pavlovian conditioning, which rules out any active participation in the examination by the small patient.

The two negative factors are

1 The subject s handling before the examina tion (placing electrodes in situ and calibrating the device) this certainly does not enable good contact to be made with the child

2 The conditioning stimulus which being a faradic shock is undeniably unpleasant and often provokes unfavourable reactions on the part of the small patient

These two negative factors generally cancel out the advantages of pavlovian conditioning

As indeed has been mentioned the faradic shock is not only not a "reward" to the small patient on the psychological plane, but even brings on negative reactions. In our own ex perience in fact, the child s agitation and subsequent crying, during both preparatory stage and first conditioning period were the principal causes of failure

On other occasions though first conditioning was established, the negative reactions arose as a result of subsequent, high-intensity faradic stimulations. Lastly to be numbered among the causes of failure was uncontrollable psychomotor agitation, often present in patients of tender age and especially in those with severe mental disorders—and any sedative treatment, besides not being readily practicable in the consulting room often does not give good re sults, as the researches by Facchini & Silimbani demonstrate P G R. cannot in fact be provoked in a state of somnolence.

Our data thus agree basically with those of previous Authors (Goldstein, Polito-Castro & Daniels Goldstein Slanger & Gottsleven Moss, Moss & Tizard Prélot & Lafon) who precisely pointed out the difficulties of examination based on P G R. study in children, and drew attention chiefly to the following causes of failure. opposition or total refusal to submit to the examina tion shyness, hyperactivity fear and some times also violent coughing at the start of the test.

As mentioned, some Authors (Schultz, Moss, Moss & Tizard) used an intense light rather than faradic shock in order to lessen these drawbacks but even with this procedure the results were not on the whole satisfactory

3 CONCLUSIONS

Data in the literature and the results of our own researches demonstrate that audiometric investigations by P G R. study in children often give unsatisfactory results and a particularly high percentage of failures. We consider for these reasons that P G.R study should be set aside for specific cases only and that proce dures taking into account children s motivations and interests should generally have preference in audiometric investigations

CHAPTER 2

Instrumental Conditioned Reflexes

1 PREVIOUS RESEARCHES

In 1944 Ewing & Ewing conducted investigations into 2 to 3 year-old children, using a set of toys capable of attracting their attention. These were: coloured wooden rods which were slipped into holes, also coloured, cylinders which had to be fitted into different-sized hollows variously coloured bobbins which were slipped on to pins brightly coloured rings of different diameter which had to be slipped on to a stick to make up a cone

The young patient s mother or an assistant to the examiner drew his attention to one of these games. When the child seemed interested in his chosen game, an attempt was made to condition him through sound stimuli provided by a whistle or drum, or a bell behind him. He had in fact to begin playing with the toy when these auditory stimuli were sent.

This type of investigation was called Play Audiometry

But credit unquestionably goes to Dix & Hallpike for having, in 1947 introduced into clinical practice a method of instrumental conditioning for examining deaf children, which quickly claimed the attention of specialists, both for its ease in use and chiefly because it achieves excellent results.

In their investigations Dix & Hallpike used device called peep-show This is basically a screen upon which are projected images or figures capable of gaining the attention or amusing the patient (animated cartoons, fairy-tale characters, etc). The projector is linked up to a pushbutton and an audiometer The sounds can be routed to a speaker when the audiometric examination is performed in free field, or straight to the patient's ear by an earphone when it is wanted to explore the two auditory apparata separately The circuit is regulated so that when the sound is present in the earphones or speaker it is enough to depress the pushbutton in order for the figures to appear on the screen. Conversely when there is no sound stimulus the image will not appear on the screen even though the child depresses the pushbutton.

To carry out the investigation the patient must be made to understand that in order to see the figures on the screen, he has to depress the pushbutton as soon as he hears the sound stimulus If the child is well conditioned he will not even attempt to use the pushbutton during the silent periods, whereas he will depress it with varying frequency and force when he hears the sounds. This will give the examiner proof that the stimulus has been perceived.

The audiometric thresholds can thus be established for the various frequencies, and an audiogram plotted.

According to some makers, conditioning can be facilitated by introducing a further prop this would be a weak red-violet lamp placed above the screen, which lights up only when the figures appear

The majority of Authors however disclaim the usefulness of this prop which can undoubtedly create confusion at the conditioning stage

It is to be briefly underlined that clear differences exist between instrumental conditioned reflexes (I.C.R.) and the pavlovian.

I.C.R., such as those utilized for instance

in investigations conducted with the peep-show *make use of a skeletal muscular response se curely bound up with activity of the cortical* nerve centres, and thus controlled by the will. Pavlovian conditioned reflexes, however which are exploited typically in audiometry based on P G R. study are generally visceral reactions attendant upon stimuli of the autonomic ner vous system and certainly not influenced by the patient s will

Since audiometry based on use of the peep-show exploits study of the reflexes involving active participation by the subject, it is also called semi-objective

Various Authors, persuaded of the usefulness of this type of audiometry have suggested nu merous modifications to Dix & Hallpike s orig-inal equipment in order to improve and chiefly promote responses of the small patients The basic purpose of these different techniques is to create situations that better accord with the child s *motivations More specifically different* attractive objects are placed at the young pa tient s disposal that enable him to start a game The *sound stimulus is practically one of the* elements of this game, and thus capable of arousing his interest In other words reaction to the auditory stimulus is part of the game to the child, while for the examiner it is proof that he hears the stimulus

We briefly recall here the principles of various modifications proposed for the Dix & Hallpike peep-show

1 Denmark used as a reward a roundabout with coloured horses on the stage of a toy theatre His examinations were performed both in free field and earphone

2 Guilford & Haug introduced a device which they called Pediacumeter This is a toy theatre with seven puppet heads that pop out one at a time as a result of the pressure the patient can exert upon a button

3 Portmann & Portmann (1953) used a projector with transparencies figuring characters drawn from popular fairy tales or at any rate known to the children

4 Barr used wooden fit in toys similar to those of Ewing & Ewing (cf page 17), the difference being that he made use of auditory stimuli provided by an audiometer

5 Statten & Wishart utilized an animated cartoon projected on to a screen set up on the facade of a dolls house the cine projector was inside the house

6 Schimizu & Nakamura found useful figures that were projected on to a large screen set up behind a partition of the room (lantern slide test)

7 Green thought of using some of the bat tery-driven toys now widely available He stud-ied the pup-show in particular This is a small mechanical puppy that went through move-ments when the child depressed the button on hearing the tone

8 Herzon used a device similar to that of Schimizu & Nakamura the Photoaudio Con ditioning Machine This is basically a projector operating in a small movie auditorium

9 *Ghirlanda proposed using a kaleidoscope* visible through a small window This is set in motion when the pushbutton is depressed

These audiometric techniques are generally recommended for children aged between 2 and 5

2 PERSONAL RESEARCHES

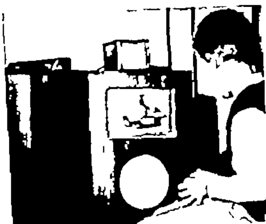
(a) Investigation method

As mentioned, various Authors have been in terested in I C R. audiometry and have put for ward different investigation procedures. These, however do not differ substantially from one another though varying in detail During recent years in the course of our researches we have tried various methods in an attempt to establish the advantages and drawbacks of each of them in relation also to previous Authors experience

Among these methods—which essentially en-able all to achieve satisfactory results—the one put forward by *Green appears specially ad* vantageous in view of the simplicity of the de-vices it requires besides the lack of difficulty *in carrying out the investigation.*

As stated Green used a small mechanical





Figs 3 4 Audiometric examination by free-field I.C.R. study using show with projector for transparencies. This show can also be used for C.O.R. study

puppy (pup-show) for conditioning the child when the patient depresses the button, the puppy's eyes light up and it does some jumps at the same time.

This game brought about fear reactions in many children, thereby hindering continuation of the research. We therefore thought it useful to replace Green's puppy with other toys, chosen from those most interesting and familiar with a view not only to overcoming this drawback but to stimulating the child's interest more and keeping it alive.

To be borne in mind in this connection is that suspicion and fickleness are the character disturbances most frequently met with in young deaf patients. These often make first contact between examiner and patient rather difficult. Using toys that all children know undoubtedly facilitates this contact, while their variety allows the examination to be prolonged until a complete audiogram is obtained

In our investigations we preferred to use either a screen upon which animated cartoons and colour transparencies figuring characters drawn from the better-known fairy tales were projected (Figs. 3 and 4) or mechanical

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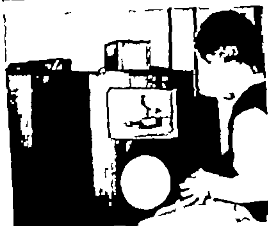
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Various Authors persuaded of the usefulness of this type of audiometry have suggested numerous modifications to Dix & Hallpike s original equipment in order to improve and chiefly promote responses of the small patients The basic purpose of these different techniques is *to create situations that better accord with the* child s motivations More specifically different attractive objects are placed at the young patient s disposal that enable him to start a game The sound stimulus is practically one of the elements of this game and thus capable of arousing his interest. In other words reaction to the auditory stimulus is part of the game to the child while for the examiner it is proof that he hears the stimulus

We briefly recall here the principles of various modifications proposed for the Dix & Hallpike peep-show

1 Denmark used as a reward a roundabout with coloured horses on the stage of a toy theatre His examinations were performed both *in free field and earphone*

2 Guilford & Haug introduced a device which they called Pediacumeter This is a toy theatre with seven puppet heads that pop out, one at a time as a result of the pressure the patient can exert upon a button

3 Portmann & Portmann (1953) used a projector with transparencies figuring characters drawn from popular fairy tales or at any rate known to the children

4 Barr used wooden *fit-in toys similar* to those of Ewing & Ewing (cf page 17), *the difference being that he* made use of auditory stimuli provided by an audiometer

5 Statten & Wishart utilized an animated cartoon *projected on to a screen* set up on the facade of a dolls house the cine projector was inside the house

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7 Green thought of using some of the battery-driven toys now widely available. He studied the pup-show in particular This is a small mechanical puppy that went through movements when the child depressed the button on hearing the tone

8 Herzon used a device similar to that of Schimizu & Nakamura the Photoaudio Conditioning Machine This is basically a projector *operating in a small movie auditorium*

9 Ghirlanda proposed using a kaleidoscope visible through a small window This is set in motion when the pushbutton is depressed

These audiometric techniques are generally recommended for children aged between 2 and 5

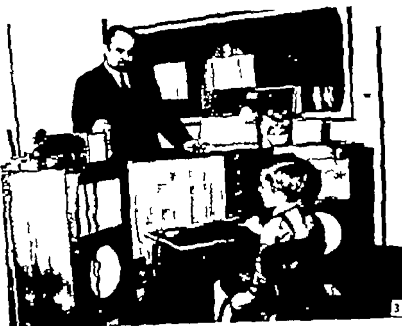
2 PERSONAL RESEARCHES

(a) *Investigation method*

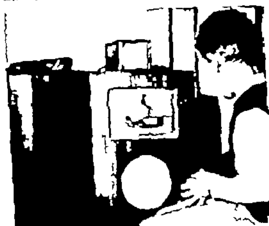
As mentioned, various Authors have been interested in I C.R audiometry and have put forward *different investigation procedures* These however do not differ substantially from one another though varying in detail During recent *years* in the course of our researches, we have tried various methods in an attempt to establish the advantages and drawbacks of each of them, in relation also to previous Authors experience

Among these methods—which essentially enable all to achieve satisfactory results—the one put forward by Green appears specially advantageous in view of the simplicity of the devices it requires, besides the lack of difficulty in carrying out the investigation

As stated Green used a small mechanical



Figs. 3–4. Audiometric examination by free-field I.C.R. study using a show with projector for transparencies. This show can also be used for C.O.R. study



puppy (pup-show) for conditioning the child, when the patient depresses the button, the puppy's eyes light up and it does some jumps at the same time.

This game brought about fear reactions in many children, thereby hindering continuation of the research. We therefore thought it useful to replace Green's puppy with other toys, chosen from those most interesting and familiar with a view not only to overcoming this drawback but to stimulating the child's interest more and keeping it alive.

To be borne in mind in this connection is that suspicion and fickleness are the character disturbances most frequently met with in young deaf patients. These often make first contact between examiner and patient rather difficult. Using toys that all children know undoubtedly facilitates this contact, while their variety allows the examination to be prolonged until a complete audiogram is obtained.

In our investigations we preferred to use either a screen upon which animated cartoons and colour transparencies figuring characters drawn from the better-known fairy tales were projected (Figs. 3 and 4), or mechanical

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1 The overall proportion of positive results was 76.2% This result is therefore particularly satisfactory

2. Indicative results were obtained only in a very small number of patients i.e., 8 6% of examined cases

3 The percent number of cases from which useful audiometric data were drawn rises progressively as patients' ages increase whereas failures were rather infrequent in children over 3 years of age, the percentage of negative results remained relatively high in those of lower age In subjects between 2 and 2 ½ years of age particularly this proportion reached 50% of examined cases.

(c) Considerations

As we see, numerous Authors have used I.C.R. study in audiometric investigations into children. Table 3 gives some data from the literature relative to the percentage of positive results attainable by such investigations into children of varying ages.

The results of our own researches agree essentially with those of earlier Authors, as regards both the progressive percent increase of successes in relation to rise in the age of patients and the high incidence of positive results in the groups over 3 years of age

Our observations (Table _) show too, that the percentage of indicative results obtained with I C R study is very low This is readily explained when the characteristics of the investigation method are borne in mind It is evident in fact that the child under test, either understands the relatively simple mechanisms underlying the method and correctly performs the conditioned-reflex movements, enabling the audiogram to be found, or he is not conditioned, and the examination fails

Table 3. *Results obtained by various Authors through I C.R. study*

Percentage of cases wherein positive results were achieved

Author	Age of examined patients (years)				
	5–6	4–5	3–4	2–3	1 2
Barr (1955)	92	81	71	41	
Sutton & Wishart (1956)	90	93	79	43	7
Haug & Guilford (1960)	96	94	82	47	12
Motta, Facchini & D'Auria		90	82	49	

The highest percentage of indicative results was had in the group of very young children whose restlessness, fatigue or other failure factors already taken into account by us for P G.R., lessened the probabilities of success in the test.

We must point out, finally the impossibility of conducting investigations by the above method into children who have severe visual defects We found this to be the case with two children in whom severe auditory damage was combined with a congenital cataract. Both pathological processes had arisen through measles suffered by the mother during the first months of pregnancy Investigation through P G.R. study enabled the hearing threshold to be established with considerable accuracy in these two cases.

3 CONCLUSIONS

Audiometric investigations based on I.C.R. study afford remarkably satisfactory results in children, especially when they are implemented with appropriate skills. The percentage of satisfactory indicative or positive results is noticeably high—around 86%—in children over 3 years old. In lower-aged patients this proportion lowers progressively and drops even to 31% in the group under 2 ½ years of age. It is therefore expedient that more suitable techniques be employed for these patients.



Fig 9 To train the child to react with the right movements to auditory stimuli, his mother or a social assistant can teach him to arrange skittles in a given order at each drum beat.

It is advisable for the child to keep his hand a little away from the pushbutton so as to prevent him from making too wide and complicated movements This is especially to be borne in mind with smaller children

Once conditioning is achieved the threshold for the various frequencies is to be sought by reducing the stimulating sound intensity by 10 dB at a time

When it is found that a frequency is not heard even at maximum intensity provided by the audiometer it is advisable also to test the neighbouring frequencies, since there may be islands" in correspondence with these wherein the sound stimulus is heard.

Sometimes a satisfactory conditioning of the child cannot be achieved during the first session In such event it is preferable rather than tire or irritate him to suspend the examination and repeat it later—even after only a few hours If despite this precaution the examination cannot be completed, it will be expedient before persisting further in the tests to instruct the parents in showing the child how to handle different educational toys (blocks fit in cylinders, etc) after an intense sound stimulation

As an instance the child s mother will be advised to have him arrange skittles in a certain order He will place one skittle at a time as soon as mother gives one beat to a drum (Fig. 9) The drum will first be held in front of the child so that he associated the visual with the auditory stimulus. Then the visual stimulus must be removed, the drum being placed behind the child and the auditory stimulus sent, taking care that he does not see his mother s hand movement as she beats the drum

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The investigation can in any case be completed another time repeating the examination in earphones when the child has learnt to cooperate with the examiner

(*b*) Results

With the equipment and by the techniques described we examined 151 subjects aged between 2 and 5 Our results are given in Table 2

Table 2. *Results obtained from 151 patients examined by way of I C R study*

Age (years)	No cases	Results: Negative n	%	Indicative n	%	Positive n	%
2½	16	8	50.0	3	18.7	5	31.3
2½–3	23	6	26.1	3	13.0	14	60.9
3–4	60	7	11.6	4	6.7	49	81.7
4–5	52	2	3.8	3	5.8	47	90.4
Total	151	23	15	13	8.6	115	76.2

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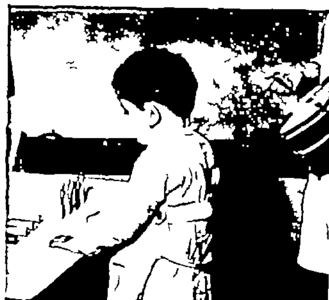


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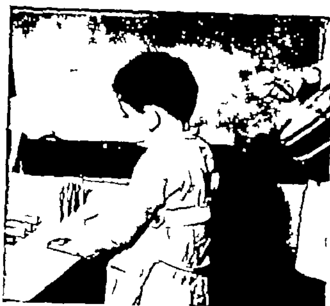


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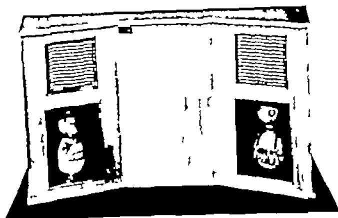
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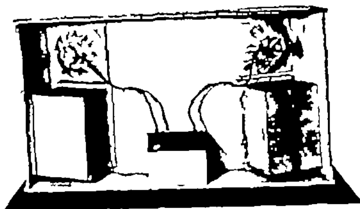


Fig 10 (A–B). The Suzuki & Ogiba theatre for audiometric examinations by way of C.O.R. study Note the position of controls and speakers at the rear

C.O.R. which is more readily established in the child because it involves no co-ordinated activity of the limbs, but merely very simple head movement.

Suzuki & Ogiba examined 115 subjects, obtaining the results given in Table 4 As this table shows, the percentage of positive results rises progressively with age up to 3 years But as the age goes up further a drop is noted in this percentage Indeed, the reward of dolls lighting up is not enough for older children in other words, their interest in it quickly flags and this prevents completing the investigation.

Table 4 *Results obtained by Suzuki & Ogiba in 115 patients by way of C.O.R. study*

Age (years)	No cases	Results: Negative		Indicative		Positive	
			%				
<1	13	8	61.5	0	0	5	38.5
1–1½	19	0	0	3	15.7	16	84.3
1½–2	15	0	0	3	20.0	12	80.0
2–2½	23	1	4.3	5	21.7	17	74.0
2½–3	29	0	0	1	3.4	28	96.6
3	16	0	0	5	31.2	11	68.8
Total	115	9	7.8	17	14.8	89	77.4

– PERSONAL RESEARCHES

( ) Investigation method

The results achieved by Suzuki & Ogiba unquestionably demonstrate the validity of their method for audiometric investigations into children under 3 years old. Above this age the Authors, also, noted that the method gives less satisfactory results.

In the course of our own investigations we

CHAPTER 3

Conditioned Orientation Reflexes

1 PREVIOUS RESEARCHES

As we have reported, the audiometric techniques described in the foregoing do not allow sufficiently demonstrative data to be obtained in children under 3 years of age

Indeed both P G.R. study and investigations based on I C.R. *study give rise to a particularly* large number of failures in these subjects as we ourselves have noted

Credit goes to two Japanese, Suzuki & Ogiba for having in 1960 invented an investigation method enabling the number of these failures to be reduced and satisfactory audiometric curves to be plotted in a very high percentage of cases

This method is based on the finding that children when subjected to a sudden light stimulus, turn their heads instinctively towards the light source This is evidently a wholly unconditioned orientation reflex It can however be conditioned by making a sound stimulus precede the luminous one a conditioned orientation reflex (C O R) can thus be achieved for *the auditory stimulus*

The following is the procedure in practice

A light source and a speaker are placed on the patient s right and left side respectively A sound stimulus is first routed on a speaker *and after about 1 sec—still from the same* side—a light stimulus the subject then turns his head towards the sources of the stimuli After these stimulations have been repeated several times, the auditory stimulus only is utilized It is then found that the patient likewise turns his head towards the sound source

To obtain this C O R Suzuki & Ogiba endeavoured to make the light stimulus conform more to the child s interests so that it con*stituted a reward They therefore built a small* theatre (Fig. 10) and fitted a speaker connected to an audiometer to each end In a recess beneath each speaker a plastic doll was placed which could be intensely illuminated by a lamp *located inside the recess*

The controls were set up behind the theatre so that the tone could be routed either to the right or the left speaker and the dolls below lit up simultaneously or otherwise

The child sitting on his mother s lap was placed at the centre of the theatre, at about 50 cm (20 inches) away from it, with his eyes at the level of the dolls The tone was sent into a speaker at an intensity definitely heard by the patient and after 1 sec, the doll below was lit up The same was done on the opposite side After repeating the operation three or four times on both sides of the theatre the child was conditioned, and one proceeded with a simple auditory stimulation The young patient s head turning towards the sound source was proof to the examiner that the stimulus had been heard

The examination was generally performed on four frequencies, the auditory stimulus intensity being reduced 10 dB at a time If a good conditioning was determined the threshold for each of the examined frequencies could be established with relative ease

Ultimately the investigation method put forward by Suzuki & Ogiba does not substantially differ as to theoretical premisses, from that based on I C R study but it lends itself better to researches on smaller children, since it uses

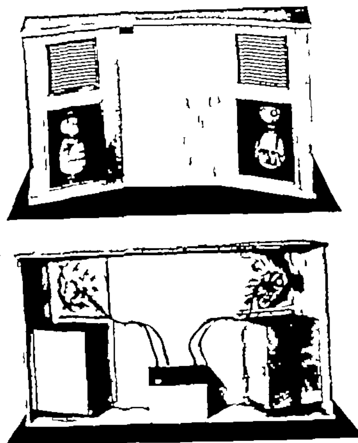


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1–1½	19	0	0	3	15.7	16	84.3
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2–2½	23	1	4.3	5	21.7	17	74.0
2½–3	29	0	0	1	3.4	28	96.6
>3	16	0	0	5	31.2	11	68.8
Total	115	9	7.8	17	14.8	89	77.4

– PERSONAL RESEARCHES

( ) Investigation method

The results achieved by Suzuki & Ogiba unquestionably demonstrate the validity of their method for audiometric investigations into children under 3 years old. Above this age the Authors, also, noted that the method gives less satisfactory results.

In the course of our own investigations we

CHAPTER 3

Conditioned Orientation Reflexes

1 PREVIOUS RESEARCHES

As we have reported the audiometric techniques described in the foregoing do not allow sufficiently demonstrative data to be obtained in children under 3 years of age

Indeed both P G R. study and investigations based on I C R. study give rise to a particularly large number of failures in these subjects, as we ourselves have noted

Credit goes to two Japanese, Suzuki & Ogiba, for having in 1960 invented an investigation method enabling the number of these failures to be reduced and satisfactory audiometric curves to be plotted in a very high percentage of cases

This method is based on the finding that children when subjected to a sudden light stimulus, turn their heads instinctively towards the light source This is evidently a wholly unconditioned orientation reflex It can however be conditioned by making a sound stimulus precede the luminous one a conditioned orientation reflex (C O R) can thus be achieved for the auditory stimulus

The following is the procedure in practice

A light source and a speaker are placed on the patient's right and left side respectively A sound stimulus is first routed on a speaker and after about 1 sec—still from the same side—a light stimulus the subject then turns his head towards the sources of the stimuli After these stimulations have been repeated several times, the auditory stimulus only is utilized It is then found that the patient likewise turns his head towards the sound source

To obtain this C O R Suzuki & Ogiba endeavoured to make the light stimulus conform more to the child's interests, so that it constituted a reward They therefore built a small theatre (Fig. 10) and fitted a speaker connected to an audiometer to each end. In a recess beneath each speaker a plastic doll was placed which could be intensely illuminated by a lamp located inside the recess.

The controls were set up behind the theatre so that the tone could be routed either to the right or the left speaker and the dolls below lit up simultaneously or otherwise

The child sitting on his mother's lap was placed at the centre of the theatre, at about 50 cm (20 inches) away from it, with his eyes at the level of the dolls The tone was sent into a speaker at an intensity definitely heard by the patient and after 1 sec, the doll below was lit up The same was done on the opposite side. After repeating the operation three or four times on both sides of the theatre the child was conditioned and one proceeded with a simple auditory stimulation The young patient's head turning towards the sound source was proof to the examiner that the stimulus had been heard

The examination was generally performed on four frequencies the auditory stimulus intensity being reduced 10 dB at a time If a good conditioning was determined the threshold for each of the examined frequencies could be established with relative ease

Ultimately the investigation method put forward by Suzuki & Ogiba does not substantially differ as to theoretical premisses, from that based on I C R study but it lends itself better to researches on smaller children since it uses

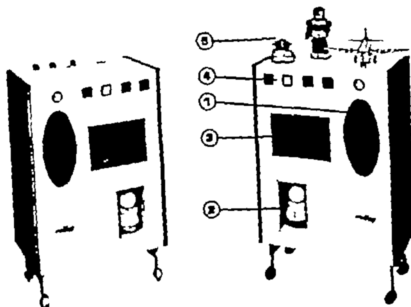


Fig. 12. The Amplifon O 9 Infant Audiology Unit for audiometric investigation via I.C.R. and C.O.R. study. 1. Speaker. 2. Recess with doll for C.O.R. study by the Suzuki & Ogiba technique. 3. Screen for projecting colour transparencies, (for C.O.R. study in older children. I.C.R. study as in the peep-show when the patient controls display of the transparencies by pushbutton linked with the projector. 4. Set of coloured lights (white, green, red ...) for C.O.R. study or to distract patient's glance should it tend to fix upon the opposite-side screen or doll during C.O.R. study. 5. Mechanical toys for I.C.R. study.

the lateral bodies there is, besides the doll, a screen upon which colour transparencies can be projected (Fig. 12).

Depending on patients age and reactive capacity we used either Suzuki & Ogiba's theatre or our own, i.e. Suzuki & Ogiba's for children under 2 years old, for 2 to 3 year-olds, Suzuki & Ogiba theatre or more frequently our own modification, depending on the patient's reactions; our own theatre for children over 3 and in all cases where attention on the dolls exhausted too quickly.

We proceeded as follows in our investigations:

1. The patients were placed in front of the show at a distance of about 30 cm, when the Suzuki & Ogiba equipment was used (Figs. 13 and 14), about 1.70 m, when using our own device (Figs. 15, 16 and 17).

The smaller children were generally held on their mother's or an assistant's lap, the older ones sat in a chair.

2. An auditory stimulus was routed consisting of a tone with frequency and intensity definitely heard by the subject. Immediately about 1 sec after the doll was lighted up or the film and transparencies were projected on to the screen.



Fig. 13. Audiometric examination of 22-month-old by C.O.R. study using the Suzuki & Ogiba theatre.



Fig 11 Show for C.O.R. study Colour transparencies on two screens above the speakers are the reward. Either of the light sources above the screens can distract the child's glance when it fixes upon the opposite-side screen. The show is also utilizable for I.C.R. study in which case the projector is linked to a push-button which depressed by the patient when a sound stimulus is routed, causes images to appear on the screen (cf Figs. 3 and 4).

often had occasion to find that such a draw back can be had even in children under 3 years of age Not infrequently in fact, did we seen patients between 2 and 3 quickly losing interest in the dolls of the Suzuki & Ogiba theatre and this prevented our completing the audiometric examination.

With this in mind we thought it expedient to modify for children over 2 years old the investigation technique put forward by these Authors, by enriching the reward The interest of these subjects in the dolls, in fact, runs out rapidly and the examination should therefore be brief Consequently the number of auditory stimuli that can be routed are insufficient for finding a complete audiogram.

Thus, as our own, Facchini, described in a previous work we used a theatre somewhat larger than the Japanese authors (Fig. 11) It had at each end a speaker and a screen upon which colour transparencies and animated cartoons could be shown by a movie projector located behind The operator was able to route the sound into one of the speakers and show the image simultaneously on the corresponding screen by means of a system of mirrors controlled by a lever The child was then conditioned by using an undeniably more effective reward than dolls The young patient's interest in the colour transparencies and films was indeed very lively and the examination could thus be made longer

Besides proving useful for various 2 to 3 year-old children, this investigation method enabled us to establish the hearing threshold in different older children, between 4 and 5 years of age More specifically these were generally (*a*) either cases where conditioning of instrumental reflexes could not be established owing to character disturbances varying degrees of intelligence deficiency or other reasons, or (*b*) patients with motor deficiencies in the limbs which did not permit using investigation techniques based on I C.R study

Our own theatre could also be used as a peep-show for I C R. study and in fact be linked up with a pushbutton which the small patient operated and set the projector working. It was thus possible in certain cases to change over readily from a C O R. examination to an investigation based on I C R study (Figs. 3 and 4)

Amplifon recently built a theatre for us that takes our findings and experiments over several years of research into account. This is a device similar to Suzuki & Ogiba's but in each of

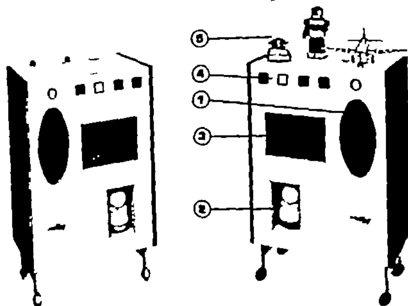


Fig 12. The Amplifon G 9 Infant Audiology Unit for audiometric investigation via I C.R. and C.O.R. study 1 Speaker 2 Recess with doll for C.O R. study by the Suzuki & Ogiba technique. 3 Screen for projecting colour transparencies, affording C.O R. study in older children, I C.R. study as in the peep-show when the patient controls display of the transparencies by a pushbutton linked with the projector 4 Set of coloured lights (white, green, red) for C.O R. study or to distract patient' glance should it tend to fix upon the opposite-side screen or doll during C.O.R. study 5 Mechanical toys for I C.R. study

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Table 5. Results obtained for 130 patients examined through C.O.R. study

(n)	No. cases	Results					
		Negative		Indicative		Positive	
	1	4	19.0	6	28.6	11	52.4
	26	3	11.5	8	30.8	15	57.7
	32	3	9.4	7	21.9	22	68.7
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But the possibilities offered by the two methods are not equal. C.O.R. study does not enable an audiogram to be established for the two single ears, whereas this can be done through I.C.R. study if patients submit to a sufficiently prolonged examination.

These data however demonstrate that in the older children, the two methods integrate one another. Either must be chosen by the examiner allowing not only for the opportunities it offers, but the particular reactivity, co-operation and character attitudes of the child under test.

It was for this reason that we contrived and had Amplifon build an apparatus that allows either investigation method to be adopted (Fig. 12). More specifically, this is a show similar to the Suzuki & Ogiba one. On each of its sides, in addition to the speaker and recess with doll provided with suitable illumination, there is a screen upon which colour transparencies figuring known classical fairy-tale characters can be projected. There are also mechanical toys. Provided, too, are light sources of various colours for distracting the child in the event of his attention being fixed on one of the sides or in special cases, for conditioning the orientation movements. This show affords ready C.O.R. study and yet allows I.C.R. also to be studied, since the appearance of colour images on the screen can be controlled by a pushbutton operated by the child, as in a peep-show.

Embodying within a single unit the devices needed for investigating by way of conditioned reflexes—orientation and instrumental—is undeniably of great advantage to the examiner who is thereby able, after a few attempts, to approach with ease the method to be adopted ... individual case.

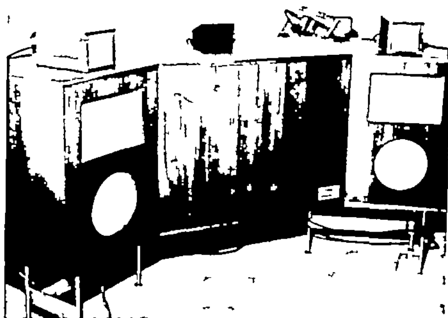


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Fig 16 Audiometric examination of a year old by C.O.R. study using a show with screens displaying colour transparencies.

leave doubt as to the actual existence of the orientation reflex.

4 Conditioning obtained, the sound stimulus was routed without following it with the visual, and turning of the patient's head towards the sound source was observed The operation was repeated, reducing the sound-stimulus intensity progressively by 10 dB at a time so as to establish the threshold for each frequency

5 In order to be sure of the extent of the tone range of sounds which had apparently not been heard in that they gave rise to no orientation reflex, these were routed at the maximum intensity afforded by the audiometer in rapid succession so as rule out possible reactions on the part of the subject. The threshold of the highest frequency heard by the patient was confirmed in addition. The following was the procedure in practice

If for instance, the last reflex reaction had been had for a 1 000 c s frequency sound at 90 dB, sound stimuli of 4 000, 3 000 and 000 c at 170 dB and of 1 000 c s a 90 dB were routed in quick succession. If in effect sound stimuli of frequencies over 1 000 c were not perceived, it was found that the child turned his head quickly towards the sound source when the last auditory stimulus was emitted.

These skills are unquestionably important for correctly defining residual hearing, necessary for fitting the patient possibly with a hearing aid and for the approach to his speech training



Fig 17 A 3-year-old examined audiometrically by C O R. study using a show with screens on to which colour transparencies are projected.

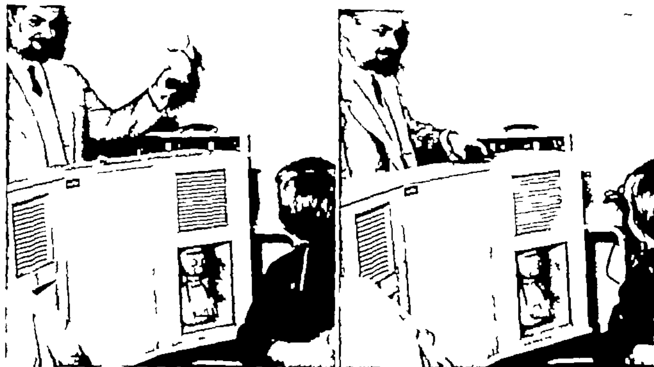


Fig 14 Audiometric examination by C.O R. study using the Suzuki & Ogiba theatre. We prefer first to investigate one side then the opposite But after a few stimulations the child's glance generally tends to fix upon the side where the sounds originate This prevents continuing the study of orientation reflexes to this side The drawback can be overcome by attracting patient s attention to a small toy getting him to turn his head towards the opposite side.

3 The operation was repeated several times until achieving the conditioning of head turning movements or orientation on the sound source

We always took care in this connection to check eye-ball as well as head-orientation movements, since these can take on considerable importance in some cases where turning of the cephalic extreme being barely perceptibl may



F g 15 A -year-old examined audiometrically by C.O R. study *using a show with screens for projecting colour transparencies.*

Table 5 *Results obtained for 130 patients examined through C.O.R. study*

Age (years)	N cases	Results					
		Negative		Indicative		Positive	
1–1½	21	4	19.0	6	28.6	11	52.4
1½	26	3	11.5	8	30.8	15	57.7
2–2½	32	3	9.4	7	21.9	22	68.7
½–3	31	2	6.5	5	16.1	24	77.4
3–4	20	0	0	3	15.0	17	85.0
Total	130	12	9.2	29	22.3	89	68.5

of these Authors, however the percentage of satisfactory results remained high even in our group of patients over 3 years old. Indeed, the positive results reached 85% in these subjects and there were no failures.

(c) **Considerations**

Our own observations above all demonstrate the importance of C.O.R. study in audiometric investigations into children under 3 years of age

This investigation method indeed appears to be decidedly more advantageous in these patients than the one based on I.C.R. study affording a much higher percentage of positive results, a a comparison between Tables 5 and clearly shows.

Our studies also demonstrate that when suitable modifications are brought to the examination method and technical equipment put forward by *Suzuki & Ogiba, the percentage of* positive results remains ery high even in patients over 3 years old In these cases it is essential during investigations to provide the small patient with a "reward that is more satisfying to him and holds his interest better than the original Suzuki & Ogiba theatre dolls.

For this reason, for investigations into children a little older we modified the Suzuki & Ogiba device by replacing the dolls with two screens upon which colour transparencies or animated cartoons were projected.

This technique often appears to be useful also for children aged – ½ to 3 It is at any rate essential for the over 3-year-olds. In this connection, of course, reactions differ appreciably in the various subjects in relation also to their psycho-intellectual development.

It is in any case interesting to note that in subjects aged between 3 and 4 the percentage of positive results with audiometry based on C.O.R. study is somewhat higher than is to be found with I.C.R. study

But the possibilities offered by the two methods are not equal. C.O.R study does not enable an audiogram to be established for the two single ears, whereas this can be done through I.C.R. study if patients submit to a sufficiently prolonged examination.

These data however demonstrate that in the older children, the two methods integrate one another Either must be chosen by the examiner allowing not only for the opportunities it offers, but the particular reactivity co-operation and character attitudes of the child under test.

It was for this reason that we contrived and had Amplifon build an apparatus that allows either investigation method to be adopted (Fig. 12). More specifically this is a show similar to the Suzuki & Ogiba one On each of its sides, in addition to the speaker and recess with doll provided with suitable illumination, there is a screen upon which colour transparencies figuring known classical fairy-tale characters can be *projected*. There are also mechanical toys. Provided, too, are light sources of various colours for distracting the child in the event *of his attention being fixed* on one of the sides or in special cases, for conditioning the orientation movements. This show affords ready C.O.R. study and yet allows I.C.R. also to be studied, since the appearance of colour images on the screen can be controlled by a pushbutton *operated by the child*, as in a peep-show

Embodying within a single unit the devices needed for investigating by way of conditioned *reflexes*—orientation and instrumental—is undeniably of great advantage to the examiner *who is* thereby able after a few attempts, to approach with ease the method to be adopted in each individual case.

We draw attention, finally to the expediency often met with in our small patients—especially when displaying hearing handicaps—of conducting investigations first into one side then into the opposite one, rather than both at once.

To be pointed out above all is that the normal hearing child has a certain facility for correctly locating the direction of a sound, even when the source is shifted from right to left, and vice versa But the child with hearing handicaps often displays considerable stiffness in reactions and tends always to turn in the direction of the first stimulus This kind of response is of course more apparent when the patient has marked differences in hearing capacity between the two ears There are undoubtedly also deaf children who after brief training tend to orientate correctly on the direction of the two sound stimuli. But very often during the first consulting room examination bilateral conditioning engenders confusion in the small patient and he consequently gives wrong responses as to the sound direction Moreover in an attempt to earn the reward he frequently tends to turn his head to the right and left before the sound stimulus is routed He thus becomes agitated and his behaviour is dispersive for the purposes of the test.

To overcome these drawbacks—and bearing in mind that the normal C O R technique is based on bilateralness of the stimuli—we attempted to condition patients on one side only introducing a disturbance visual stimulus In practice our procedure was as follows

(i) If the Suzuki & Ogiba theatre was used, the examiner at the start of investigation placed himself in front of the subject, beyond the theatre itself (Fig. 14) He kept a small rubber toy or railway engine handy He then proceeded to condition the child on the doll of one side—the left, *for instance* At a given moment the patient fixed his glance on this side in order to see the doll light up The examiner then distracted his attention by showing him the rubber toy or engine which were slowly moved from left to right of the theatre stage Interested in the new toy the child turned his head to the right The auditory signal was routed at this point and, if it was heard, the child was seen to turn his head to the left again

We used different toys to distract the patient, ensuring that these were not more stimulating than the Suzuki & Ogiba lighted-up doll We also took care to execute the movements tending to distract the child *at a certain speed so* that his interest remained concentrated on the doll This was to avoid his no longer co-operating in the investigation through attention being drawn to the new toy

(ii) If it was a case of older patients, for whom the modified show equipped with projector was utilized, the child was conditioned by getting him to look at the film or colour transparencies on the screen of one side only the left, for example At a given point, when he had fixed his glance on the screen waiting for the animated cartoons to appear the examiner lit up a particularly bright lamp on the right, screened by frosted glass (Figs 11 12 4 15 16 and 17) situated on the surface of the device itself. The subject was thereby distracted, and instinctively turned to the right. At this juncture the auditory signal was given on the left and any head turning toward this side was observed.

(*b*) Results

Our investigations utilizing the procedures we have described, involved 130 subjects from 1 to 4 years old

Our results are given in Table 5 They are classified as negative, indicative and positive by the same criteria as in page 15

When the results we obtained through C O R study are taken overall one first finds that the failure percentage by this method is really low indeed not coming up to 10% of examined cases.

The data in Table 5 further demonstrate that the percentage of positive results rose progressively with the age of patients, as Suzuki & Ogiba also found As opposed to the findings

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To be pointed out above all is that the normal hearing child has a certain facility for correctly locating the direction of a sound, even when the source is shifted from right to left, and vice versa. But the child with hearing handicaps often displays considerable stiffness in reactions and tends always to turn in the direction of the first stimulus This kind of response is of course more apparent when the patient has marked differences in hearing capacity between the two ears There are undoubtedly also deaf children who after brief training tend to orientate correctly on the direction of the two sound stimuli But very often during the first consulting-room examination, bilateral conditioning engenders confusion in the small patient and he consequently gives wrong responses as to the sound direction Moreover in an attempt to earn the reward he frequently tends to turn his head to the right and left before the sound stimulus is routed He thus becomes agitated and his behaviour is dispersive for the purposes of the test.

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(i) If the Suzuki & Ogiba theatre was used, the examiner at the start of investigation placed himself in front of the subject beyond the theatre itself (Fig. 14) He kept a small rubber toy or railway engine handy He then proceeded to condition the child on the doll of one side—the left, for instance At a given moment the patient fixed his glance on this side in order to see the doll light up The examiner then distracted his attention by showing him the rubber toy or engine which were slowly moved from left to right of the theatre stage Interested in the new toy the child turned his head to the right The auditory signal was routed at this point and if it was heard, the child was seen to turn his head to the left again

We used different toys to distract the patient, ensuring that these were not more stimulating than the Suzuki & Ogiba lighted up doll We also took care to execute the movements tending to distract the child at a certain speed so that his interest remained concentrated on the doll This was to avoid his no longer co-operating in the investigation through attention being drawn to the new toy

(ii) If it was a case of older patients, for whom the modified show equipped with projector was utilized, the child was conditioned by getting him to look at the film or colour transparencies on the screen of one side only the left for example At a given point, when he had fixed his glance on the screen waiting for the animated cartoons to appear the examiner lit up a particularly bright lamp on the right, screened by frosted glass (Figs 11 12, 4 15 16 and 17) situated on the surface of the device itself. The subject was thereby distracted, and instinctively turned to the right. At this juncture the auditory signal was given on the left, and any head turning toward this side was observed.

(b) **Results**

Our investigations utilizing the procedures we have described involved 130 subjects from 1 to 4 years old

Our results are given in Table 5 They are classified as negative, indicative and positive by the same criteria as in page 15

When the results we obtained through C O R study are taken overall one first finds that the failure percentage by this method is really low indeed, not coming up to 10% of examined cases

The data in Table 5 further demonstrate that the percentage of positive results rose progressively with the age of patients, as Suzuki & Ogiba also found As opposed to the findings

Table 5 *Results obtained for 130 patients examined through C O R. study*

Age (years)	No. cases	Results Negative		Indicative		Positive	
1–1½	21	4	19.0	6	28.6	11	52.4
1½–2	26	3	11.5	8	30.8	15	57.7
2–2½	32	3	9.4	7	21.9	22	68.7
2½–3	31	2	6.5	5	16.1	4	77.4
3–4	20	0	0	3	15.0	17	85.0
Total	130	12	9.2	29	22.3	89	68.5

of these Authors, however the percentage of satisfactory results remained high even in our group of patients over 3 years old. Indeed, the positive results reached 85% in these subjects, and there were no failures.

(c) Considerations

Our own observations above all demonstrate the importance of C.O.R. study in audiometric investigations into children under 3 years of age.

This investigation method indeed appears to be decidedly more advantageous in these patients than the one based on I.C.R. study affording a much higher percentage of positive results, as a comparison between Tables 5 and 2 clearly shows.

Our studies also demonstrate that when suitable modifications are brought to the examination method and technical equipment put forward by *Suzuki & Ogiba*, the percentage of positive results remains very high even in patients over 3 years old. *In these cases it is es*sential during investigations to provide the small patient with a "reward" that is more satisfying to him and holds his interest better than the original Suzuki & Ogiba theatre dolls.

For this reason, for investigations into children a little older we modified the Suzuki & Ogiba device by replacing the dolls with two screens upon which colour transparencies or animated cartoons were projected

This technique often appears to be useful also for children aged 2½ to 3 It is at any rate essential for the over 3-year-olds. In this connection, of course reactions differ appreciably in the various subjects, in relation also to their psycho-intellectual development.

It is in any case interesting to note that in subjects aged between 3 and 4 the percentage of positive results with audiometry based on C.O.R. study is somewhat higher than is to be found with I.C.R. study

But the possibilities offered by the two methods are not equal. C.O.R. study does not enable an audiogram to be established for the two single ears, whereas this can be done through I.C.R. study if patients submit to a sufficiently prolonged examination.

These data however demonstrate that in the older children, the two methods integrate one another Either must be chosen by the examiner allowing not only for the opportunities it offers, but the particular reactivity co-operation and character attitudes of the child under test.

It was for this reason that we contrived and had Amplifon build an apparatus that allows either investigation method to be adopted (Fig. 12) More specifically this is a show similar to the Suzuki & Ogiba one. On each of its sides, in addition to the speaker and recess with doll provided with suitable illumination, there is a screen upon which colour transparencies figuring known classical fairy-tale characters can be projected. There are also mechanical toys. Provided, too are light sources of various colours for distracting the child in the event of his attention being fixed on one of the sides or in special cases, for conditioning the orientation movements. This show affords ready C.O.R. study and yet allows I.C.R. also to be studied, since the appearance of colour images on the screen can be controlled by a pushbutton operated by the child, as in a peep-show

Embodying within a single unit the devices needed for investigating by way of conditioned reflexes—orientation and instrumental—is undeniably of great advantage to the examiner who is thereby able, after a few attempts, to approach with ease the method to be adopted in each individual case.

We draw attention finally to the expediency *often met with in our small patients*—especially when displaying hearing handicaps—of conducting investigations *first* into one side, then into the opposite one rather than both at once

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(*b*) Results

Our investigations, utilizing the *procedures we* have described, involved 130 subjects from 1 to 4 years old

Our results are given in Table 5 They are classified as negative indicative *and positive* by the same criteria as in page 15

When the results we *obtained through C O R* study are taken overall one first finds that the failure percentage by this *method is really low* indeed, not coming up to 10% of examined cases

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For this reason, for investigations into children a little older we modified the Suzuki & Ogiba device by replacing the dolls with two screens upon which colour transparencies or animated cartoons were projected.

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Embodying within a single unit the devices needed for investigating by way of *conditioned* reflexes—orientation and instrumental—is undeniably of great advantage to the examiner who is thereby able, after a few attempts, to approach with ease the method to be adopted in each individual case

3 CONCLUSIONS

Our observations show that the most suitable investigation method for establishing the audiometric curve in children under 4 years old is the one based on C O R. study

This investigation has to be conducted by adapting the technique to the child's age, his mental development and psychomotor reactions More specifically the theatre designed by Suzuki & Ogiba perfectly meets the needs of the investigation in smaller children Over 3 years of age, however the child s interest in the Suzuki & Ogiba dolls tends quickly to exhaust itself and this often necessitates bringing the reward" into line by more interesting games For this reason we used a show similar to Suzuki & Ogiba s, but wherein colour transparencies or animated cartoons can be projected

In older children—and especially those between 3 and 4 years of age—it can be established each time depending on their reactions, whether it is preferable to use this show or I C.R study Both techniques in fact enable a high percentage of positive results to be achieved although each has its advantages and disadvantages More specifically

1 C.O.R. study affords positive good or indicative results in the totality of examined cases. It is not possible however to explore the auditory capacity of the two ears singly by this method

2 I C.R study though giving rise to a larger number of failures, nevertheless allows an audiogram to be plotted for each ear in cases where good conditioning can be established and the examination prolonged sufficiently

We therefore proposed a device and had it built by Amplifon for audiometric investigations into children that enables both techniques to be performed thereby giving the examiner the opportunity to select the more appropriate one, taking examination demands and patients capacity for co-operation into account

Appendix

OBJECTIVE CONDITIONED-REFLEX AUDIOMETRY IN MULTI HANDICAPPED CHILDREN

1 Premises

Audiometric investigations into children unquestionably present sizable difficulties if, besides any hearing disability there are psycho-intellectual alterations or handicaps in skeletal motility or yet impairment of different sensorial apparata. The existence of pathological situations of this kind can make the child's conditioning very complicated indeed, and hinder the audiometric investigations we have described in the foregoing.

In these conditions the researches that aim at establishing the existence or otherwise of an auditory deficiency cannot generally be conducted within the short space of time demanded by a consulting-room investigation as defined at the beginning of this work. It is usually necessary to proceed with a lengthy series of observations over a period of time and with the co-operation of the neurologist, psychologist, teacher and phoniatrician.

Only in this way will effective collection be made of all the data affording precise identification of the various sensorial, intellectual, mental and motor handicaps presented by the patient, and accurate framing of the clinical case. It will then be possible to direct the patient to centres where he will be given the most efficient help and correct training be implemented.

We ourselves are often faced with typical situations in current practice i.e., we are asked either to examine children whose hearing is certainly impaired and who have other handicaps, and it is wished to know the extent of their auditory deficiency or to establish whether or not certain multi-handicapped subjects displaying psycho-intellectual disturbances have impaired auditory function, and what part this has played in retarding their mental development.

We took four groups of subjects into account in our survey. We think the investigation procedures adopted in each of these groups and the technical skills employed in the individual cases described, give a clear enough idea both of the difficulties involved in such researches and of the facilities we have for overcoming them.

2 Personal researches

The groups seeming most representative to us are made up of:

(a) Subjects with disturbances in symbolic function
(b) Mentally handicapped subjects with low I.Q
(c) Spastic or athetosic subjects
(d) Subjects multi-handicapped through foetal virosis contracted by mother during early months of intrauterine life

(a) *Subjects with disturbances in symbolic function*

These are subjects who, through alteration of certain cortical centres and resultant impairment of integrating capacity present difficulty or outright impossibility in organizing all the psychomotor activities underlying speech.

These patients have been defined as aphasic oid (Myklebust; Coen Rosen etc.) or even dysgnosic (Prélot & Lafon). Audiometric investigation into these cases always presents considerable difficulty.

The main difficulty the audiologist has to tackle is that children with such handicaps cannot usually manage to understand the prob-

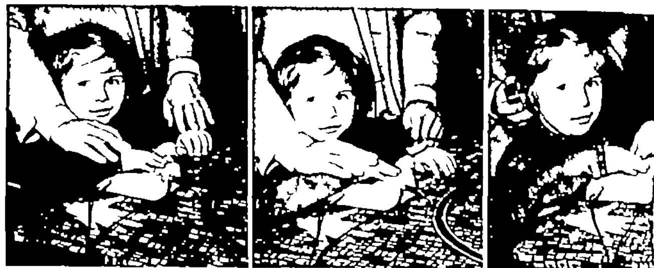


Fig 18 Audiometric examination by I C.R study of a child with symbolic function disorders. To get her to understand the relation between electric train movements and auditory stimulus, conditioning was done over successive periods. The bone vibrator was first placed in contact with the palm, then on the wrist and finally on the mastoid, at which stage the patient was conditioned by a sound-tactile stimulus. Conditioning established the vibrator was replaced by an earphone thereby suppressing the tactile stimulus.

lem of I C R. audiometry Consequently it is impossible to establish correct conditioning.

These patients in fact, generally fix their whole attention upon a toy but do not link its movement possibilities with the tone present in the speaker and with pressure on the button They therefore remain wholly inert during the various stages of the examination, or press the button connected with the toy whether the sound is present in the speaker or not. Only in rare cases particularly favourable to the investigation is it observed that any time he hears the tone the child watches the toy hoping for it to move spontaneously This reaction is undoubtedly in itself information useful to the examiner

Generally therefore it is found that the tone by air conduction is not a stimulus of adequate intensity for these patients i e such as to draw their attention. Even when the hearing loss is slight they often fail to react also to stimuli of marked intensity (90–100 dB on frequencies between 125 and 500 c/s)

Frisina & Bernero pointing out the scant possibilities of establishing conditioning in many of these patients of pre-school age with the use of pure tones by air conduction proposed applying a bone vibrator to the fit-together toy they used for examining children by play-audiometry Their procedure was as follows.

A 500 c/s tone at 50–70 dB was first routed into the vibrator and the child under test was trained to perceive the tactile stimulus and move the toy to build a tower every time the stimulation was given. The vibrator was then transferred to the child's palm, his wrist and lastly to his mastoid. At this stage the tactile stimulus was necessarily combined with a sound stimulus which the patient received by bone conduction The examination then proceeded by placing the child's palm on an audiometer earphone into which a 500 c/s tone at 100 dB was routed. Once the child was conditioned by this new tactile-vibratory stimulation the examination continued by transferring the earphone to the ear The child could then be observed to react correctly also to the sound stimulus alone

After using the Frisina & Bernero technique for about two years we chose to modify it with a view to bettering its results More specifically in *free field examinations* we employed I C.R. study with either of the following methods, depending on the small patient s reactions.

Method I After placing the bone vibrator under the child's palm we conditioned him so he would be induced to depress the button linked to the toy each time the tone was routed into the vibrator We then placed the vibrator in contact with his wrist, and finally on the mastoid (Fig. 18). The child was con

ditioned by the sound as well as the tactile stimulus during the latter stage. When sure that the conditioning was well established, we removed the vibrator and routed the auditory stimulus alone on the speaker. While enabling us to achieve good results in several patients, this method sometimes presented drawbacks, i.e. (*a*) The small patient was at times afraid to keep his hand resting on the bone vibrator even when before starting the investigation the examiner showed him how harmless it was by placing his own hand upon it;
(*b*) Once the tactile-vibratory stimulus was removed and the tone routed by air conduction, the child often remained indifferent to the new stimulation.

Method II To overcome these drawbacks we chose the following procedure for some patients. The child put his palm directly on speaker into which grave-frequency sound was routed (we generally used a 250 or 500 / tone at 80 dB). H was then induced to depress the pushbutton operating the toy each time he perceived the tactile-vibratory stimulus and heard the sound (Fig. 19). The child's conditioning was achieved more easily by using these different stimuli simultaneously and, chiefly he could be made to understand that an association existed between stimuli and toy movements. Once a satisfactory conditioning was obtained, the speaker was placed in front of the child, and the examination proceeded with the sound stimulus alone. Often when he heard the tone, the child sought tactile confirmation of its presence, and brought his hand on to the speaker to ascertain its vibration.

I our view this second modification offers several advantages over the method based on bone vibrator and in fact it enables stimulus by air conduction to be combined with the tactile-vibratory stimulus used initially. It is thus possible to pass more readily on to the second stage of the investigation, where the air-conduction stimulus only will be used it voids any particular fright reaction, in small children as ell

As Frisina & Bernero mentioned, in the case of *examinations in earphone* it is undeniably ery useful to associate in the child's mind a relationship between tactile-vibratory and sound stimulus. To do this it is expedient first to place the child's palm on the earphone into which the tone is routed (preferably a 500 c s sound at 90 dB), so that he becomes conditioned to react by depressing the button that sets the toy in motion each time he is stimulated.

Some cases studied by us far better illustrate the possibilities and limitations of these audiometric investigation methods.

Case 31 Age 6 years. Born at term. Weight 3.8 kg. Jaundice of moderate entity at birth, lasting 5–6 days. No exchange transfusion.



Fig. 19 A low I.Q. patient examined audiometrically by I.C.R. study. As in Fig. 18 he was first conditioned by a tactile-sound stimulus, getting him to place his palm on the speaker. Conditioning established, the tactile stimulus was suppressed and the examination proceeded in free field.

Neurological findings: considerable general hypotonia and difficulty in feeding during first month. Extreme retardation in walking (started at 30 months). Neurological examination revealed hyperreflexia and bilateral hint of a Babinski.

E.E.G.: signs of distress of deep origin, with irritative aspects.

At the age of 6 his I.Q. by Borelli-Oleron test fluctuates between 0.55 and 0.60. Marked degree of phrenasthenia. Extreme difficulty in structuring very simple forms. No possibility of graph, this is at the prefiourage stage.

Speech: first words spoken when 3 / years old. Child says "babbo, mamma, nonna, bimbo (papa, mama, grandma, baby) and few other words of incomprehensible phonetic structure.

Audiometric investigation: enormous difficulty encountered in conditioning child with pure tones; besides poorly understanding the problem of relations between sound stimulus and toy movement, he presents

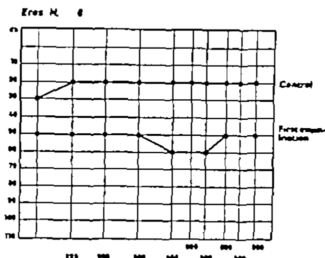


Fig 20 (*Eros M* aged 6.) Patient aphasic through neonatal jaundice. Audiometric examination was by I.C.R. study in free field. There is an evident flucting hearing. The first examination revealed a hearing *loss of about 50 dB Control after 10 days* established the loss to be very modest

flucting-hearing, giving conflicting responses as to the hearing threshold for sound stimuli, even when spaced 40–50 dB from one another On the whole however the data obtained lead to suppose that a moderate hearing capacity exists.

After repeated tests, initially getting his hand to rest *upon the speaker then conducting the investiga* tion in free field his conditioning was managed.

The first audiogram so executed enabled the hearing threshold to be located at a level of about 50 dB A control after 10 days established the patient's hearing *loss* to be very moderate and not exceeding 20 dB (Fig. 20).

Present behaviour subject still extremely distracted, paying no particular attention to the world around him. He seems able at times to understand not-too-complicated speech like andiamo via (let's go) or andiamo dalla nonna (let's go to grandma's) at *others he reacts to no oral stimulus*. He does not supplement his lack of speech with gestures. If he does not manage to get what he wants, he is taken by attacks of temper accompanied by crying.

The child is under training at the Bologna Municipal *Orthophonic Centre His possible scholastic* progress is unassessable at the moment

Silvana T Age 9 years. Born at term. Weight 3 2 kg. Severe jaundice from materno-foetal incompatibility *of the Rh system, breaking out 4 days after birth and* lasting 40 No exchange transfusion.

Neurological findings. somnolence and grave difficulty in feeding during first months after birth A high degree of general hypotonia also present whereby patient succeeded *in sitting up at 10–11 months and* began walking at 14 months.

E.E.G moderate diffuse irritative signs.

At the age of 9 her I.Q defined by the Terman test is 0 60. *Speech and arithmetic tests*, even for 5 year-olds, were negative. The Wisc blocks tests was moderately well resolved in relation patient's age. The Bender test revealed her to be sufficiently structured for her age

Speech. the child presented severe retardation in speech development. Up to the age of 4 she uttered few words with evident dyslalia. Her education had been approached wrongly on the assumption that more-or less severe deafness existed, she was first sent to a school for the hard-of-hearing. In this environment, where she was scarcely impelled to talk, *her speech suffered a further blockage. She remained* practically mute for many months. Despite her mother's assertion that the child's hearing was normal, the teacher and specialist were unable to realize that this was so. The specialist, in particular was deceived by her passive behaviour during audiometric examinations, and by the wrong responses she gave. The teacher on the other hand, influenced by long experience of untrue or inaccurate anamnestic data furnished by relatives, was also led into error by the child's particular behaviour

Audiometric investigation the patient presented *marked flucting-hearing. During the first tests she* did not even respond systematically to stimulations by 1 000, 2 000 and 3 000 c/s tones routed at 100 dB leading one to presume a severe deficiency on the conversation voice frequencies. Subsequent in*vestigations, conducted by the procedures we have* described, enabled the patient to be conditioned satisfactorily and established her hearing to be practically normal (Fig. 21)

Present behaviour when it was realized that the patient's hearing was normal she was transferred to

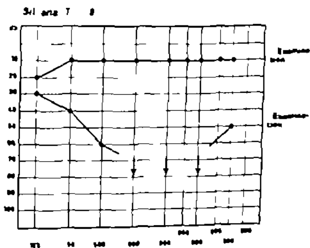


Fig 21 (*Silvana T* aged 9) Patient aphasic through severe neonatal jaundice *from materno-foetal incom*patibility of Rh system. Audiometric examination was by free-field I.C.R. study The findings first led a severe auditory deficiency to be presumed Subsequent examinations by appropriate techniques disclosed a nearly normal *hearing capacity*

a school for the mentally handicapped. Her speech has developed, but it remains severely dyslalic and imprecise. She says "papa" for "zio" (uncle), "sera" (evening) for "mattina" (morning). She learns a word and easily forgets it. She often breaks off the conversation through not knowing the necessary words for completing it.

Her behaviour is still scarcely socialized, unstable and dependent. She is incapable of dressing or undressing herself, often collides with desks and seems absent.

(b) *Mentally handicapped subjects with low I Q*

Many of the skills employed in subjects with symbolic-function disorders, and described in the foregoing paragraph, can often also be advantageous for low I.Q. patients to achieve a good conditioning of instrumental reflexes and so use the study of these to find the audiogram.

It is to be pointed out at once, however, that the study of instrumental reflexes and generally of play-audiometry is only possible when patients with I.Q. over 50 are examined. According to some authors (Prélot & Lafon) and to our own experience, the attempts to establish conditioning of instrumental reflexes in children with I.Q. lower than this value are generally attended by failure and it is therefore definitely preferable to use C.O.R. study in these cases.

By adopting these approaches we could resolve—sometimes at one session only—cases that might have taken several days of observation or induced the examiner to postpone the test for a few years.

We must nevertheless mention that, in conflict with every technical forecast, the gravity of the handicaps is sometimes such as to render fruitless all attempts at establishing the characteristics of the possible hearing deficiency.

Sauro E. Age 13 years. Born at eighth month. Weight 1.8 kg.

Severe jaundice from materno-foetal incompatibility of the Rh system, arising 10 days after birth, with clear signs of intolerance to mother's milk and convulsive episodes.

Neurological findings: frequent convulsive attacks

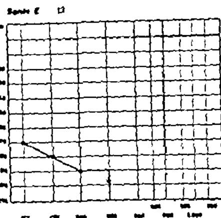


Fig. 22 (Sauro E., aged 13.) Patient with gravely diminished I.Q. through severe neonatal jaundice from materno-foetal incompatibility of Rh system. Audiometric examination by free-field C.O.R. study revealed severe auditory deficiency.

during first 6 months of life. Severe alteration in trophism and motoricity. Walking began at about 6 years of age.

The objective neurological examination revealed divergent strabismus. The upper limbs, especially the right, presented in flexion, and lower limbs in an attitude of slight adduction. Also bilateral Babinski. Walking spastic in type.

E.E.G. under barbiturate sleep: no signs with clear pathological significance noted.

I.Q. undefinable.

Audiometric investigations: on 14.1.64 an examination was attempted with the usual toys for studying I.C.R. The patient displayed absolute resistance to any form of co-operation. He not only wouldn't push the button but even refused to look at the toys. The test was suspended after several tries because he kept his face hidden on his mother's shoulder. After about an hour had elapsed, an attempt was made to perform an examination through C.O.R. study.

During this investigation the patient remained permanently in an attitude of opposition, with face hidden on his mother's shoulder. But when grave tones, 125 to 500 c/s at 90–100 dB, were routed into the speaker, he raised his head slightly and tried to look at the colour projections over his mother's shoulder. If the examiner watched him, however, he quickly hid his face and shut his eyes.

The audiometric tests via C.O.R. study were repeated on 18.1.64. The patient, though remaining on the defensive, looked at the film when tones from 125 to 500 c/s were routed. The existence of moderate hearing residue was thereby established in correspondence with these frequencies (Fig. 22).

Present behaviour: patient is considered irrecoverable.



Fig 23 (A–F). Audiometric examination by I C R. study of a patient with spastic manifestations. The assistant first helped him to depress the push button causing colour transparencies to appear Then, though with some uncertainty he managed to understand what movements were necessary and was able to do them by himself showing interest in the game linked with the examination.

(c) *Spastic or athetosic subjects*

Audiometric investigations into these patients may present difficulties chiefly because owing to altered functionality of certain muscular groups they are unable to execute or co-ordinate the reflex movements telling the examiner that the sound stimulus has been perceived

In *spastic subjects* with slight or moderate clinical manifestations the audiometric examination can be performed by helping the child to do the movements he should make when he hears the auditory signal

Let us take as an example the case where the investigation is done by way of I C R study the subject can be helped to push the button setting the toy in motion, provided that he hints at wanting to do so (Fig. 23)

In severer cases it will be enough to receive

even a small sign of the child's intention, and the examiner can push the button.

In *subjects with athetosic manifestations* the examination is generally very much more painstaking. As is known, movements are accentuated in these patients and they are exacerbated in all emotive situations, especially when they are required to accomplish motor actions of some precision. The considerable difficulties entailed in audiometric examination under such conditions are consequently understandable. Even with C.O.R. study the results will depend upon the gravity of spontaneous movements of the cephalic extreme. When the subject's head continually accomplishes in-co-ordinated movements, in fact, it is impossible to determine whether an orientation reflex of the head appears or not.

But at times when the tone stimulus is routed, more-accentuated athetosic movements or a series of grimaces may be had, or a smile in expectation of the reward, or a deviation of the eyeballs, reactions revealing that the stimulus has been perceived.

To be remembered, lastly, is that in cases where the patient can be given an instruction—that is, when it can be explained to him what movements he must do in the event of hearing a sound stimulus—an attempt can be made to get him to do any movement, even putting his tongue out (Finck & Finck) after each perceived tone emission.

We consider anyway that, except in cases with spastic manifestations of moderate entity it is difficult to subject these patients to consulting-room examination using objective audiometry.

It will often be necessary to have them admitted to specialized centres where they can undergo repeated investigation and prolonged observation. It will thereby be possible to gather all the information useful to any training.

Iadevaro R. Age 6 years. Born at term. Weight 3.35 kg.

Very severe jaundice few hours after birth, from materno-foetal incompatibility of the Rh system. Total exchange transfusion.

Neurological findings: subject presented grave difficulty in feeding during first three months of life. Severe neonatal hypotonia. Walking began towards 18th month. Choreo-athetosic movements, especially of the neck and upper limbs, as well as oculogyric crises, were noted at 3 years of age. At 4 walking was still uncertain, with a drunken gait.

As to phonation, he uttered no word although his mother stated he was able to understand various phonemes if scanned in loud voice.

E.E.G. subcortical abnormalities with irritative features.

I.Q. established by non-verbal tests is 0.87–0.90.

Audiometric investigations: the first researches were conducted at the age of 4. They were made difficult by the patient's extreme instability and the motor in-co-ordination he displayed.

It was managed at times to make him understand the problems linked with I.C.R. audiometry but he assigned scant value within his perceptive range to the auditory stimulation, even when very intense, and thus did not properly execute the movements for informing the examiner as to perception of the sound. Moreover, when it was succeeded in disinhibiting him, he began to overturn objects and want the toys without submitting to any test.

He showed readiness to tire in the course of investigations based on C.O.R. study.

Because of all this it was impossible either to perform an examination or to collect indicative data as to his hearing abilities.

He was nevertheless admitted to a school for the hard-of-hearing. His adaptation during the first year was especially difficult. Indeed, he paid no attention to anything, took what he wanted, satisfied his bodily needs in the midst of his companions, in the classroom without warning the teacher. This was not due to his being unable to communicate with the teacher but utter indifference as to his environment and total disinhibition.

Slowly over the space of about 2 years, there was some improvement in both behaviour and motoricity. The training exercises could thus be started.

A fresh audiometric examination revealed a hearing loss of moderate entity on the conversation voice frequency (Fig. 24).

Present behaviour: in view of the audiometric findings it was decided to fit a hearing aid. It has thus been possible to improve the patient's phonatory training.

Athos P. Age 4 years. Twin birth. Weight 2.9 kg. The twin died at 1 month.

Severe jaundice a few hours after birth, lasting 20 days.

Neurological findings: during the neonatal period there were frequent temperature rises with convulsive events. Skeletal muscle tonicity appeared to be severely diminished as from the early months; upright position was achieved at around 16 months and walking began towards the 30th month. A state of general hyperreflexia was revealed during a control examination performed at the age of 4, swallowing of saliva appeared difficult and incomplete.



Fig 23 (A–F). Audiometric examination by I C R. study of a patient with spastic manifestations. The assistant first helped him to depress the push-button causing colour transparencies to appear Then, though with some uncertainty he managed to understand what movements were necessary and was able to do them by himself showing interest in the game linked with the examination.

(c) *Spastic or athetosic subjects*

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It will often be necessary to have them admitted to specialized centres where they can undergo repeated investigation and prolonged observation. It will thereby be possible to gather all the information useful to any training.

Vladimiro R. Age 6 years. Born at term. Weight 3.33 kg.

Very severe jaundice a few hours after birth, from materno-foetal incompatibility of the Rh system. Total exchange transfusion.

Neurological findings: subject presented grave difficulty in feeding during first three months of life. Severe neonatal hypotonia. Walking began towards 18th month. Choreo-athetosic movements, especially of the neck and upper limbs, as well as oculogyric crises, were noted at 3 years of age. At 4 walking was still uncertain, with a drunken gait.

As to phonation, he uttered no word although his mother stated he was able to understand various phonemes if scanned in a loud voice.

E.E.G.: subcortical abnormalities with irritative features.

I.Q. established by non-verbal tests is 0.87–0.90.

Audiometric investigations: the first researches were conducted at the age of 4. They were made difficult by the patient's extreme instability and the motor in-co-ordination he displayed.

It was managed at times to make him understand the problems linked with I.C.R. audiometry but he assigned scant value within his perceptive range to the auditory stimulation, even when very intense, and thus did not properly execute the movements for informing the examiner as to perception of the sound. Moreover, when it was succeeded in disinhibiting him, he began to overturn objects and want the toys without submitting to any test.

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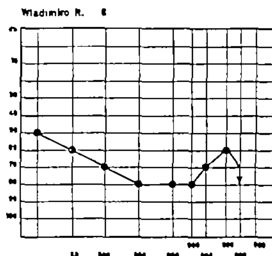


Fig 24 (*Vladimiro R* aged 6.) Subject with choreo-athetosic manifestations. Audiometric examination by I.C.R. study via earphone reveals a severe bilateral all-tone hearing loss.

E.E.G severe signs of deep distress.

I.Q and behaviour child docile and tranquil on the whole He watches what goes on around him but takes no part in his brothers games, confining himself to knocking down their toys or buildings. He is not aggressive he is almost indifferent to sounds or noises An attempt to perform the Borel Maisonny test met with complete failure.

Speech is practically non-existent

Audiometric investigation. examination only practicable through C O R. study Patient's responses uncertain and inconsistent, and merely enable one to suppose that severe deafness exists (Fig. 25)

Present behaviour owing to the considerable hearing disability and grave psycho-intellectual deficiency the patient has been sent to a centre for the multi handicapped

(d) Subjects multi-handicapped through foetal virosis contracted by mother during early months of intrauterine life

We think it important to discuss our observations in some subjects who as a result of rubeolar infection suffered by the mother during the early months of pregnancy presented, besides a usually very severe disability in hearing, a defect either in visual function or intelligence and at times also altered structuring of symbolic functions Performing the audiometric examination clearly presents considerable difficulties Consequently the audiologist drawing upon his own experience must know how to select the most suited technique from those we have described But there is no doubt that not infrequently the problems linked with audiometric examination are complicated due mainly to the scant co-operation patients are able to give in view of their psycho-intellectual handicaps. Moreover their visual defects often do not allow instrumental and orientation conditioned reflexes to be studied. The examiner thereby finds himself deprived of the techniques which, as we have illustrated, can provide the best results within the field of infant audiology

But there is no doubt that if he exercises particular patience in the research he can detect specific reactions by the patient as a result of the various stimulations, that will allow him to obtain an audiogram which if not accurate, is at least indicative

Indeed even when the patient cannot see the toys properly owing to ocular lesions, he can nevertheless present the conditioned reflex reactions either because he vaguely distinguishes the movements of the toys, or is able to perceive the light stimuli (To be recalled in this connection is that on both sides of the show for C O.R. study there are always intense light sources consisting either of the lamp illuminating the doll recess or independent lamps of different colours—cf page 31)

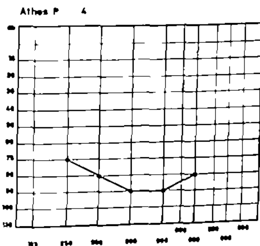


Fig 25 (*Athos P* aged 4) Subject with spastic manifestations. Audiometric examination by free-field C O R. study discloses a severe bilateral hearing loss.

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SUPPLEMENT 288

Studies of the Distribution of Cochlear Potentials Along the Basilar Membrane

BY

L. U. E. KOHLLÖFFEL

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STOCKHOLM, SWEDEN

ACTA OTO-LARYNGOLOGICA

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Studies of the Distribution of Cochlear Potentials Along the Basilar Membrane

BY

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To my Family

CONTENTS

1 Introduction

Most of our knowledge about the hydromechanical action of the inner ear we owe to the admirable work of Georg von Békésy (1960 *a*). His direct observations of the basilar membrane vibration established the travelling wave pattern of disturbance as the mode of response by which the cochlea reacts to sound. Upon tonal stimulation of the inner ear a travelling wave appears on the cochlear partition whose wavelength becomes shorter as we proceed from base to apex. The envelope of the displacement wave has a flat basal and a steeper apical slope this gives the envelope its typical asymmetrical shape. The position of the rather flat envelope maximum depends on stimulus frequency it shifts with decreasing frequency progressively from the basal towards the apical end of the cochlea. Observing the excursion of points along the membrane as a function of frequency von Békésy obtained their frequency response curves. When expressed in db per octave the typical slope values of his response curves are 6 db per octave for the low frequency and 20 db per octave for the high frequency branch

Von Békésy used in his studies stimulus frequencies of not more than 4 kHz, i.e. he worked in the apical, low frequency region of the basilar membrane. Recently the Mossbauer effect was put to use in studies of cochlear mechanics at the basal, high frequency end of the basilar membrane. Johnstone & Boyle (1967) and Johnstone (1969) working on the guinea pig obtained response curves with low frequency slope values of 12 db per octave and high frequency slope values of 70 to 100 db per octave at an SPL around 90 db. Rhode & Geisler (1969) measured in the squirrel monkey slope values of 5 to 10 db per octave on the low frequency side and 90 to 150 db per octave on the high frequency side.

It would seem that these more recent mechanical data conform better to the threshold-frequency response curves of primary auditory nerve fibres obtained by Tasaki (1954 1960) than do the curves of von Békésy However evidence compiled over the past few years on the discharge patterns of primary fibres makes it appear doubtful whether a simple relationship exists between the neural output from segments along the basilar membrane and the corresponding local mechanical input. Ingeniously devised experiments on the neural response to aperiodic stimuli (Kiang, 1965 de Boer 1969 Møller 1970) revealed a transducer mechanism with much less damping than is implied by the strongly damped response of the basilar membrane (Weiss, 1964 Flanagan, 1960 Siebert, 1962). Additionally threshold frequency response curves (tuning curves) obtained by several investigators (Katsuki et al., 1958 Kiang, 1965 Evans, 1970 *a*) are relatively narrow over a considerable part of the dynamic range and fibres respond to a wide band of frequencies only at relatively high sound pressures. In view of this discrepancy between the neural output data and the mechanical input data of the cochlear transducer as far as frequency selectivity is concerned it seems necessary to postulate a 'sharpening mechanism intervening between the mechanical displacement of the membrane and the resulting neural discharges in auditory nerve fibres. However at present mechanical data are only available for stimulus intensities of 90 db SPL or above, i.e. from that part of the dynamic range where neural tuning curves are wide. Thus there is the important question whether

output at x and H that at x_2. H and H_2 are represented as spatial impulses. Each hair-cell generator establishes a potential field according to the conductive properties of inner ear tissues and fluids. The decay of potential strength with longitudinal distance from the generating source is indicated for scala tympani by an exponential attenuation function. It can be clearly seen from the illustration that an electrode at point x_e records a potential which is the sum, or superposition of the contributions from the generators at x_1 and x_2. In the case of n hair-cell generators we obtain as expression for the electrical output at x_e.

$$CM(x_e) = \sum_{i=1} H(x_i)\,w(x_e - x_i)$$

$w(x_e - x_i)$ is a weighting factor which takes account of the signal attenuation between the point of generation x_i and the recording site x_e. We may conceive of the basilar membrane as infinitely densely packed with hair-cell operators and consequently arrive at an integral expression for the CM at x_e.

$$CM(x_e) = \int_{-\infty}^{\infty} H(x)\,W(x - x)\,dx \qquad (1)$$

Equation (1) is fundamental to the present study. It defines the relationship between the hair-cell output pattern along the basilar membrane, $H(x)$, and the resulting CM distribution along the cochlear scalae, $CM(x_e)$. $W(x - x)$ is a weighting function which brings into play the signal attenuation effective between the points x and x_e. The expression in equation (1) is a convolution integral and by analogy to linear communication theory we may call the connection between $H(x)$ and $CM(x_e)$ a 'spatial filter operation'. Hence $H(x)$ is the 'input function' and $CM(x_e)$ the corresponding output function. $W(x)$ is the spatial impulse response of the system.

Experiments to be described here were designed to examine the validity of equation (1). A suitable starting point seemed to be the study of CM distributions in response to high frequency tones. Such stimuli cause disturbances only in the basal coil of the cochlea and it seemed possible to measure very accurately and completely the corresponding CM patterns with a multi-electrode array arranged along the basal cochlea. At high frequencies the travelling waves have very short wavelengths and thus closely neighbouring hair cells are driven out of phase. One might expect therefore that phase cancellation is much more pronounced and that the spatial filter effect can be seen much better at high frequencies than at low ones.

it is permissible to simply extrapolate mechanical data to sound pressures lower than 90 db SPL where the neural response is extremely sharply tuned

Since the study by Tasaki Davis & Legouix (1952) it is well known that the excitation pattern along the basilar membrane may be investigated in an alternative way to that followed by von Békésy These authors inserted four pairs of differential electrodes into the four turns of the guinea pig s cochlea and they measured the longitudinal distribution of the cochlear microphonic (CM) in response to sinusoidal stimuli The resulting CM distributions and their dependence on stimulus frequency were qualitatively similar to von Békésy s observations on the vibratory pattern of the basilar membrane. Legouix (1965/6 1969) using the same technique, studied the CM in response to impulsive stimuli His findings are in good qualitative agreement with mechanical data the transient CM response shows the typical strongly damped oscillations with a natural frequency characteristic to the region of the basilar membrane from which an electrode is recording There is good evidence that the CM response is associated with the shearing motion at the hair bearing end of the sensory hair cells (von Békésy 1960 *a*) and that it originates in the region of the receptor pole of the hair cells (Tasaki et al 1954) That is why we shall use throughout this study the term hair-cell generator when strictly speaking we only mean the source of the CM in the organ of Corti

Thus there are basically two ways in which the excitation pattern along the basilar membrane may be studied In the one mechanical excursions are measured directly In the other measurement is made of the electrical potentials which are the consequence of the mechanical excursions The greater sensitivity inherent in the electrical method offers the advantage that the CM can be used to explore the excitation pattern in the region of lower sound pressures, i.e in the region below 90 db where the minute membrane deflections have

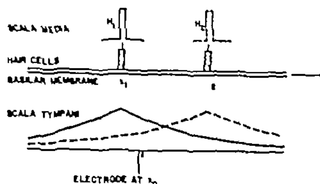


Fig 1 1 Diagram to show the interference of hair-cell outputs (*H* and *H*) in the cochlear fluids due to signal spread along the canals. Electrode at x_0 records a potential which is the sum of the attenuated contributions from hair-cell generators at *x* and *x*

not yet been resolved by mechanical methods. The disadvantage of the electrical method, however is one of data interpretation in the past there has been considerable uncertainty on the question how to evaluate exactly CM recordings taken with electrodes from the cochlear fluids. This question concerns the relationship between the potentials generated within the organ of Corti and those picked up by recording electrodes. A constructive step towards a better understanding was taken by Whitfield & Ross (1965) in a theoretical study They defined mathematically the relationship between hair-cell generator potentials and electrode outputs. According to this definition an electrode records the 'weighted sum of the electrical activity along the membrane This, it is argued is due to the fact that hair-cell potentials spread out and interfere with each other to a considerable extent in the inner ear fluids. Thus the spatial spread of signals in the cochlea was recognised to be instrumental to the quantitative interpretation of CM data and a reappraisal of CM measurements seemed appropriate

The present investigation developed logically from the mathematical formula relating hair cell output to electrode output In Fig 1 1 two hair-cell generators are schematically shown situated on the basilar membrane at points *x* and x_2 respectively Let *H* be the electrical

output at x_1 and H_2 that at x_2. H_1 and H_2 are represented as spatial impulses. Each hair-cell generator establishes a potential field according to the conductive properties of inner ear tissues and fluids. The decay of potential strength with longitudinal distance from the generating source is indicated for scala tympani by an exponential attenuation function. It can be clearly seen from the illustration that an electrode at point x_e records a potential which is the sum, or superposition of the contributions from the generators at x_1 and x_2. In the case of n hair-cell generators we obtain as expression for the electrical output at x_e.

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2 Methods

For the study of the spatial filter effect it was necessary to obtain a detailed picture of the CM distribution over a sufficient length of the cochlea. Analysis of the microstructure of CM patterns with equation (1) seemed only a promising enterprise provided accurate and reliable data about the longitudinal course of the CM were made available. The accommodation of an assembly of closely spaced electrodes allowing the detailed inspection of CM patterns was the central problem in the development of suitable techniques.

The set of recording electrodes was placed in the basal scala tympani of the guinea pig's cochlea. This site was chosen for mainly two reasons. First this part of the cochlea appeared to be spacious enough to allow the insertion of several electrodes without damage to the cochlear partition. Second the CM response to high frequency stimuli was expected to show the spatial filter effect most clearly. It was therefore imperative to execute measurements in the basal area where the response to such stimuli is known to occur.

The choice of the basal cochlea as recording site carried the advantage that the conditions of current spread have been studied most extensively for this part of the cochlear ducts (see Section 3 Weighting Function). As to the question which recording technique should be used, the differential technique with a set of paired electrodes along the cochlear partition or the simpler technique with a set of electrodes in one scala and a common reference electrode outside the cochlea, e.g. on the animal neck, there seemed to be no objection from existing evidence to using the simpler technique. For CM recordings from the upper turns of the guinea pig's cochlea most workers have preferred the differential technique, i.e. they recorded the potential difference between one electrode in scala tympani and one opposite in scala vestibuli. It has been suggested in the literature (Tasaki et al. 1952; Tasaki & Fernández, 1952) that the differential technique is superior to the alternative of measuring potentials between one electrode in scala tympani and the other on the neck. A differential pair of electrodes is supposed to record potentials only from one turn without contamination by signals arising from neighbouring turns. However Tasaki & Fernández felt that for the basal coil such a superiority did not exist and they stated: The microphonic recorded with one electrode on the neck and the other in the basal turn, either in scala vestibuli or in scala tympani, gives information as to the activity taking place in the basal turn without contamination by the responses originating in the upper turns. Recently Dallos (1969 *a*) confirmed this statement in a thorough study. Apart from the fact that the differential technique recorded potentials larger by 6 db in the basal turn he could not find any advantage over the simpler technique, i.e. one electrode in the basal scala tympani and the reference on the neck. In our case the simpler technique is preferable for mainly two reasons: 1) The technical difficulties in setting up a differential recording situation with twelve closely spaced pairs of electrodes are most probably prohibitive. 2) Cross-conduction between scala vestibuli and scala tympani via the fluid leaking out of the openings in the cochlear wall would probably be unavoidable. This would introduce uncontrollable current loops short-circuiting the cochlear partition. Thus it is clear that the disadvantages of the differential technique in

our case, outweigh the advantage of recording potentials larger by 6 db.

The problem of arranging a set of recording electrodes over a short length of the basilar membrane seemed to be most elegantly tackled by the use of a multi-electrode probe manufactured with the modern techniques of microminiaturization. Such a probe was manufactured for this project in the Lucas Research Department, Shirley Birmingham. Twelve narrow stripes of aluminium were photoetched onto a glass substrate which was cut into a rectangular shape appropriate for the insertion of the probe through a slit into scala tympani. However this approach proved unsuccessful because the probe was brittle and much too delicate to be repeatedly used in a large series of experiments. Thus very early on, during the exploratory phase of the project, the probe broke and this technique was discontinued. After this failure an alternative technique was adopted. A series of up to twelve holes was drilled into the wall of the basal scala tympani parallel to the basilar membrane and stainless steel wire electrodes were inserted into the holes. The placement of electrodes was extremely time-consuming. This method was finally abandoned in favour of the ultimate optimum technique which allowed the rapid collection of data. A multi-electrode array was constructed by gluing insulated stainless steel wires together. The elastic, ribbon-like array thus formed could be easily inserted through a slit into scala tympani alongside the basilar membrane.

SURGICAL PROCEDURE

All guinea pigs were anaesthetised with Urethane administered intraperitoneally at a dose of 1.25 g kg. Tracheotomy was performed and a short polythene cannula was inserted into the trachea. The bulla on the animal's right side was exposed in the standard latero-ventral fashion described by Tasaki & Fernández (1952). Then the animal was mounted in a stereotaxic instrument with the head rigidly clamped between the skull and the upper jaw bone in a head holder. The external auditory meatus was exposed and cut across leaving only a short 'sleeve' for the insertion of the coupling tube of the earphone.

With a number of wire hooks the tissue surrounding the bulla was carefully retracted. The bulla was opened with a dental drill and part of the bony shell was removed with a forceps. Thereafter the cochlea protruding into the middle ear cavity was conveniently accessible for surgical manipulation. With small pieces of cotton wool the surface of the cochlea was cleaned and dried.

After these preliminary operations microdissection began with the aid of a Zeiss otoscope which both illuminated and magnified the operative field. With a small, round dental burr (1/4 C. Ash & Sons) the wall of the basal 1/2 turn of scala tympani was thinned down to uniform thickness along the course of the basilar membrane. The thickness was reduced until the bony wall was almost transparent. This procedure had to be executed with utmost care. Accidental sideways slipping of the burr by only about 1 mm and subsequent injury to the retracted tissue in the immediate vicinity could cause bleeding and filling of the middle ear cavity with blood. Removal of blood clots especially from the stapedial region was very delicate. It was found empirically that guinea pigs weighing about 500 g possessed cochlear walls with the optimum mechanical consistency for this operation. Guinea pigs below this weight had cochleae too fragile and heavier animals usually had a bony shell too hard and brittle. In either extreme case it could easily happen that the cochlear shell broke during the drilling process.

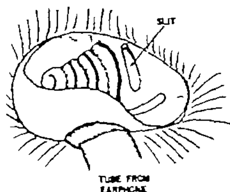
In the early experiments ('single electrode technique') a number of holes were drilled with a stiff needle into the wall of scala tympani parallel to the basilar membrane. Since it was desirable to accomplish a fairly regular spacing of the holes the needle was mounted in a heavy handle. The weight of the handle stabilized the needle once it was placed on the

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For the study of the spatial filter effect it was necessary to obtain a detailed picture of the CM distribution over a sufficient length of the cochlea. Analysis of the microstructure of CM patterns with equation (1) seemed only a promising enterprise provided accurate and reliable data about the longitudinal course of the CM were made available The accommodation of an assembly of closely spaced electrodes allowing the detailed inspection of CM patterns was the central problem in the development of suitable techniques.

The set of recording electrodes was placed in the basal scala tympani of the guinea pig s cochlea. This site was chosen for mainly two reasons. First this part of the cochlea appeared to be spacious enough to allow the insertion of several electrodes without damage to the cochlear partition. Second the CM response to high frequency stimuli was expected to show the spatial filter effect most clearly It was therefore imperative to execute measurements in the basal area where the response to such stimuli is known to occur

The choice of the basal cochlea as recording site carried the advantage that the conditions of current spread have been studied most extensively for this part of the cochlear ducts (see Section 3 Weighting Function) As to the question which recording technique should be used, the differential technique with a set of paired electrodes along the cochlear partition or the simpler technique with a set of electrodes in one scala and a common reference electrode outside the cochlea e g on the animal neck, there seemed to be no objection from existing evidence to using the simpler technique. For CM recordings from the upper turns of the guinea pig s cochlea most workers have preferred the differential technique i e they recorded the potential difference between one electrode in scala tympani and one opposite in scala vestibuli It has been suggested in the literature (Tasaki et al 1952 Tasaki & Fernández, 1952) that the differential technique is superior to the alternative of measuring potentials between one electrode in scala tympani and the other on the neck A differential pair of electrodes is supposed to record potentials only from one turn without contamination by signals arising from neighbouring turns. However Tasaki & Fernández felt that for the basal coil such a superiority did not exist and they stated 'The microphonic recorded with one electrode on the neck and the other in the basal turn either in scala vestibuli or in scala tympani gives information as to the activity taking place in the basal turn without contamination by the responses originating in the upper turns Recently Dallos (1969 a) confirmed this statement in a thorough study Apart from the fact that the differential technique recorded potentials larger by 6 db in the basal turn he could not find any advantage over the simpler technique i e one electrode in the basal scala tympani and the reference on the neck In our case the simpler technique is preferable for mainly two reasons 1) The technical difficulties in setting up a differential recording situation with twelve closely spaced pairs of electrodes are most probably prohibitive 2) Cross-conduction between scala vestibuli and scala tympani via the fluid leaking out of the openings in the cochlear wall would probably be unavoidable This would introduce uncontrollable current loops short-circuiting the cochlear partition Thus it is clear that the disadvantages of the differential technique in





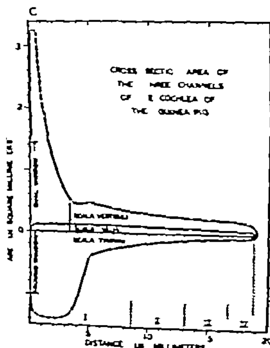


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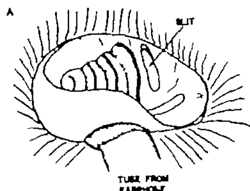
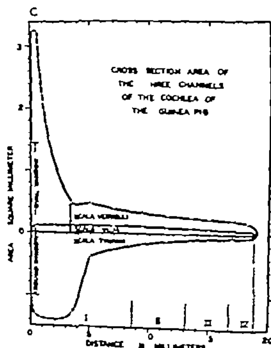




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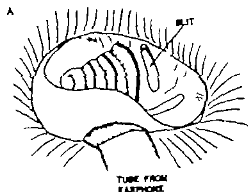
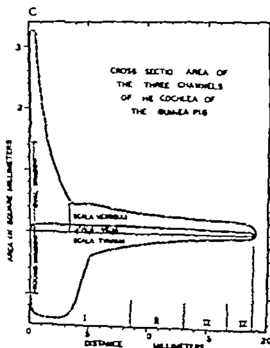




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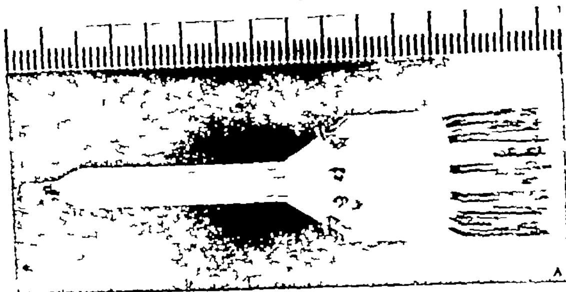
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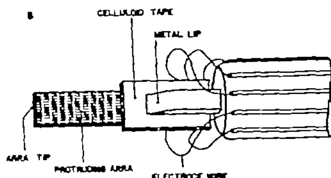


Fig 2.3 (A) Photograph of the electrode probe carrier' (scale division in mm).

(B) Schematic drawing of the tip of the electrode probe carrier' with the electrode *array mounted*.

carrier and the main shaft. It contained the electrical components necessary for the a.c. coupling between electrodes and preamplifiers. (For the measurement of cochlear d.c.-potentials this a.c.-coupler could be removed. In the experiments reported here no d.c.-measurements were undertaken and the a.c.-coupler was always in place.)

The shape of the complete electrode array holding unit represented a compromise between two partly conflicting design criteria. It was desirable to make the twelve wires leading away from the recording site as short as possible in order to minimize capacitive cross-talk, i.e. the preamplifiers had to be as close as possible to the recording site. On the other hand the preamplifiers had to be accommodated at a sufficient distance from the operative field not to obstruct the experimenter's vision.

C. Insertion of the electrode array into scala tympani

The alignment of wires was checked under the Zeiss otoscope before insertion of the tip of the electrode array into scala tympani. During the course of three to four experiments usually small pieces of polystyrene broke away from the tip of the array causing an irregular spacing of electrode terminals. By carefully cutting the tip of the array with a pair of scissors it was possible to use the remaining intact part of the array for further experiments. In this way the same array could be used for about ten successive experiments.

For the placement of the array in scala

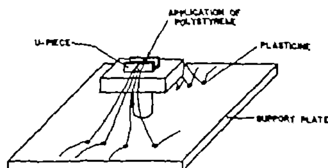


Fig 2.2 Schematic drawing of the wire assembly arrangement.

the longitudinal distribution of the CM response. The individual electrodes composing the probe were made of the same 50 μ stainless steel wire which was used in the single electrode technique. Steel wires, about 150 mm long, were slowly drawn through a bath of Bakelite Insulating Lacquer The coat so formed was then baked for not less than 20 min in an oven at 180 C. Wires were given at least three coats.

Twelve insulated wires were assembled side by side in a single plane and they were bonded together with polystyrene The assemblage was carried out under visual control (Zeiss otoscope) In order to achieve an accurate alignment of the electrodes in a single plane the wires were pulled over the edges of a U shaped piece of metal. A schematic drawing of the simple assembly arrangement is given in Fig 2.2. The procedure began by tightly pulling the first insulated wire over the U piece and fixing the wire ends on the support plate with plasticine The second wire was arranged in the same way close to the first one With a paint brush a small droplet of a polystyrene benzene solution was spread along the electrode wires over a length of about 15 mm Due to the surface tension of the liquid the wires were pulled close together The polystyrene dried rapidly and fixed the wires Then the third electrode was bonded to the second one and the manipulation was repeated until all twelve electrode wires were glued together In the event that any particular wire within the set was not aligned properly a small drop of benzene was applied so that the polystyrene softened locally This made a realignment of the wire possible

The distance between adjacent electrode wires was mainly determined by the blob-like thickenings of the insulation coating along the wires. It was possible to achieve a regular spacing of electrode wires in the array by carefully selecting wires with approximately equal blob sizes. Thus the distance between the centres of adjacent electrodes varied from *about 150 to 180 μ Since the electrode dia*meter was 50 μ this variation in distance was considered tolerable.

B. Mounting of the electrode array

One end of the assembly of wires was trimmed with scissors and the completed ribbon like array was then mounted on the electrode probe carrier' (see Fig 2.3 A) A rectangular piece of ordinary film tape was glued to the small metal lip sticking out of the carrier shaft The electrode array was bonded with Eastman adhesive 910 to this flexible tape support in such a way that about 8 mm of the ribbon like array protruded beyond the support A schematic drawing of this arrangement is given in Fig. 2.3 B The free ends of the electrode wires were embedded with polystyrene in twelve grooves cut lengthwise into the round shaft of the carrier Finally after scraping off the insulation from the terminal section with a scalpel the electrode wires were soldered to twelve small steel bars permanently attached to the upper end of the carrier The carrier was made of heat resisting Teflon to avoid melting of carrier material during soldering

The carrier was then connected to the main part of the array holding unit (see Fig 2.4) This part consisted of a round perspex shaft with twelve permanently embedded steel wires leading to twelve preamplifiers arranged at the top end of the shaft A short cylindrical piece of perspex was inserted between the probe

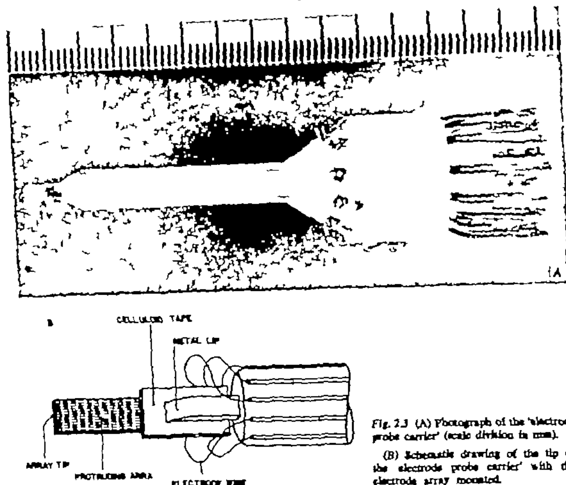


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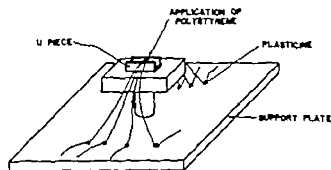


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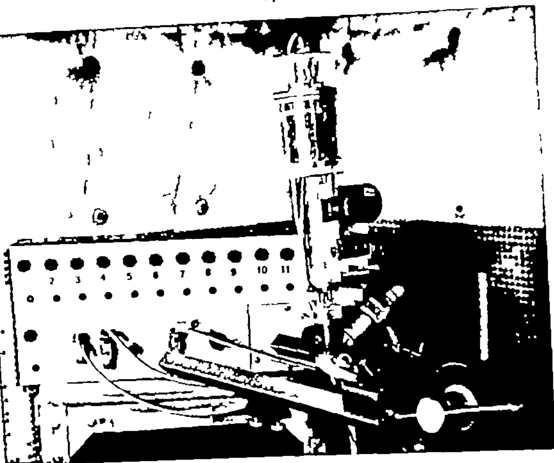


Fig. 2.5. Photograph showing the electrode array holding unit in the experimental position on the stereotaxic instrument. The array tip is inserted into the basal scala tympani of the right cochlea in the guinea pig skull. The holding unit is coupled via the ball joint to manipulator II. Preamplifiers on the holding unit are connected to the twelve main amplifiers contained in the box, shown in the background. The screened condenser earphone with the short metal tube for the acoustic coupling is displayed next to the guinea pig skull.

ELECTRICAL PROPERTIES OF THE ELECTRODE CHANNELS

It was essential for the faithful measurement of the CM distribution in the basal scala tympani that all data channels had identical electrical characteristics. In Fig. 2.7 one such channel is shown schematically representative for the whole set of the twelve parallel channels. The electrode wire was a.c.-coupled to the preamplifier. (Both a.c.-coupler and preamplifier were attached to the array holder.) The preamplifier was connected with a cable to the main amplifier stage contained in a separate box. The preamplifier in series with the main amplifier formed the "channel amplifier".

Recordings were taken between the sensing electrodes in scala tympani and an indifferent electrode on the animals neck. Hence the "internal impedance of the signal source was the sum of two components. The one component was the impedance between the basal scala tympani and animal earth which according to von Békésy (1960 *a*, p. 659) had a value of about 8 kΩ. The other component was the impedance at the electrode-perilymph interface. (The insulation coating was absent only at the cross section of the tip of the steel electrode.) This impedance amounted to ap-

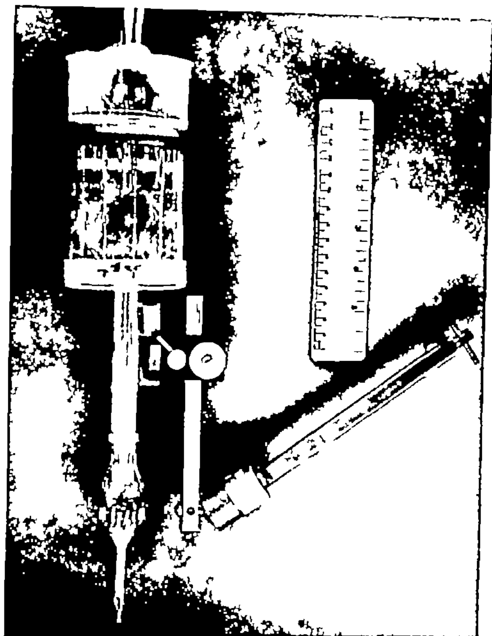


Fig 2 4 Photograph of the electrode array holding unit and preamplifiers. The small, ribbon-like electrode array is shown at the tip of the unit. The holding unit *is mounted on manipulator* I which in turn is coupled via the ball joint link (shown in the photograph) to manipulator II (shown *in* Fig. 2 5)

tympani a combination of two micromanipulators was employed (see Fig 2 4 and Fig 2.5) Manipulator I was coupled to manipulator II via a ball and-socket joint this gave sufficient freedom of movement to manoeuvre the *array tip into scala tympani through the narrow* slit in the cochlear wall The main shaft of the array holding unit was screwed to manipulator I With the ball joint unlocked manipulator I was tilted in such a way that the electrode array tip was in line with the slit in the basal turn Then the ball joint was locked and the electrode array position was adjusted in the lateral directions with manipulator II Finally the array was driven into scala tympani with manipulators I and II

D Channel connections in the 'single-electrode' technique

In the single-electrode technique all electrodes were mounted in a perspex block (see Fig. 2.6). *With short leads connection was made to* the a c.-coupler attached to the main shaft of the array holding unit. Thus in both techniques *the same channel amplifiers were employed for* the recording of potentials.

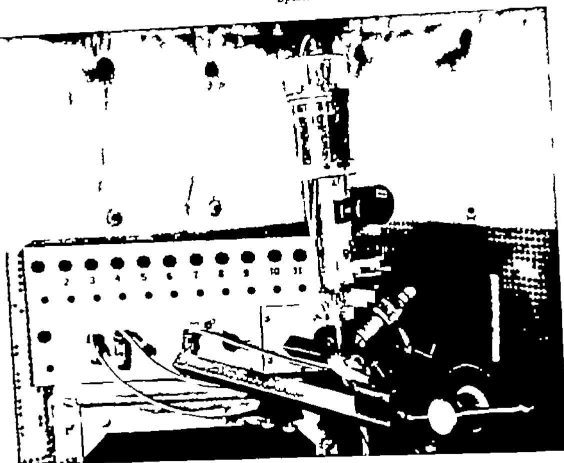


Fig 2.5. Photograph showing the electrode array holding unit in the experimental position on the stereotaxic instrument. The array tip is inserted into the basal scala tympani of the right cochlea in the guinea pig skull. The holding unit is coupled via the ball joint to manipulator II. Preamplifiers on the holding unit are connected to the twel e main amplifiers contained in the box, show in the background. The screened condenser earphone with the short metal tube for the acoustic coupling is displayed next to the guinea pig's skull.

ELECTRICAL PROPERTIES OF THE ELECTRODE CHANNELS

It was essential for the faithful measurement of the CM distribution in the basal scala tympani that all data channels had identical electrical characteristics. In Fig. 2.7 one such channel is shown schematically representative for the whole set of the twelve parallel channels. The electrode wire was a.c.-coupled to the preamplifier (Both a.c.-coupler and pre amplifier were attached to the array holder.) The preamplifier was connected with a cable to the main amplifier stage contained in a separate box. The preamplifier in series with the main amplifier formed the channel amplifier'

Recordings were taken between the sensing electrodes in scala tympani and an indifferent electrode on the animals neck. Hence the 'internal impedance of the signal source was the sum of two components. The one component was the impedance between the basal scala tympani and animal earth which according to von Békésy (1960 *a* p. 659) had a value of about 8 kΩ. The other component was the impedance at the electrode-perilymph interface. (The insulation coating was absent only at the cross section of the tip of the steel electrode.) This impedance amounted to ap-

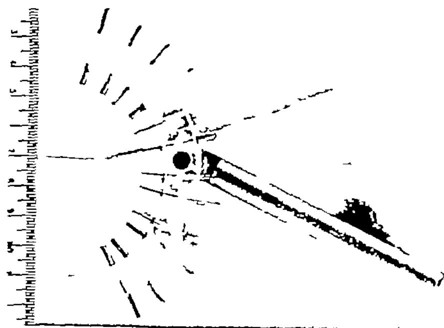


Fig. 2.6 Photograph of the ring-shaped perspex block for the mounting of electrodes in the single electrode technique. The handle of the electrode wire (shown in the inside of the ring) is pushed into the complementary brass cylinder embedded in the block. The electrode wire thus mounted is then connected with a lead (shown on the outside of the ring) to a channel amplifier (scale division in mm).

proximately 30 kΩ. Due to the high input resistance of the preamplifiers (22 MΩ) there was no danger that slight differences in the "internal impedance" of the twelve channel sources would distort the measurement of the CM pattern. In addition the high input resistance of the preamplifiers served a further purpose. It was important not to introduce with the sensing equipment current loops of high conductivity which could alter the conductivity pattern present in the cochlea. The high input impedance of the data channels prevented such

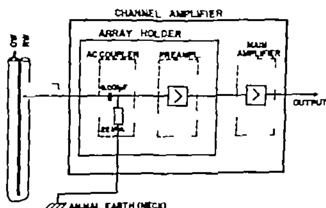


Fig. 2.7 Block diagram of one channel amplifier representative for all twelve channel amplifiers. (The schematic drawing on the left shows the twelve electrodes of the array inserted into scala tympani. RW – round window, OW – oval window.)

a disturbance of the current distribution in scala tympani.

All twelve transistorized amplifiers were built identically and they had the same flat frequency response from 0 to 20 kHz. The 3 db cut-off frequency was 40 kHz. Since electrodes were a.c.-coupled to the amplifiers a lower 3 db cut-off frequency of about 10 Hz was introduced. Thus over the frequency range (500 Hz to 20 kHz) used in the study the frequency response of the amplifiers was flat. The gain factor of the channel amplifiers was set at 500. However, despite careful adjustment there remained a slight difference of maximally 2% between the gain factors of different channels.

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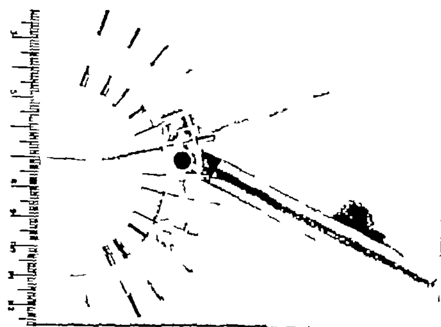


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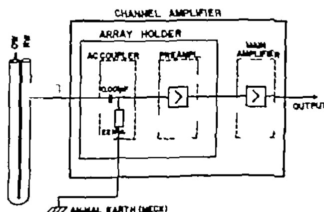


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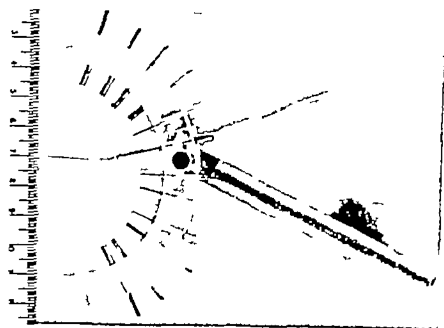


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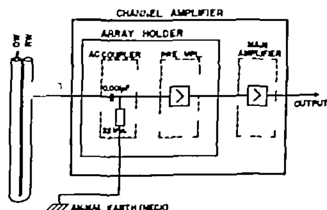


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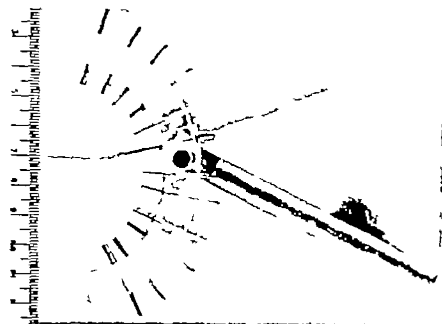


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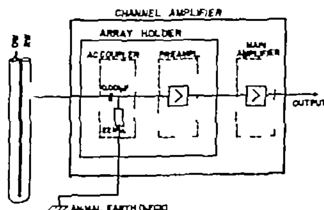


Fig. 27. Block diagram of one channel amplifier representative for all twelve channel amplifiers. (The schematic drawing on the left shows the twelve electrodes of the array inserted into scala tympani, *RW* = round window, *OW* = oval window.)

01	20	14 50				
1	0 1682E 02	0 1705E 02	0-2354E+02	0 1372E 01	0 0	316 4
2	0 1317E 02	0 1577E 02	0 2055E 02	0 1313E 01	3 7	300 1
3	0 77 1E 01	0 1509E+02	0-1694E+02	0-1229E+01	16 4	332 0
4	0 3311E 01	0 1310E 02	0 1351E 02	0 1131E 01	29 4	345.8
5	0 6675E 00	0-1005E 02	0 1008E 02	0 1005E 01	35 6	356.2
6	0-1947E 01	0 2676E 01	0 3318E 01	0 5196E 00	7 5	323 9
7	0 3370E 01	0 3510E 00	0 2415E 01	0 5324E 00	53 7	260 7
8	0 5630E 01	0-1517E+01	0-5831E+01	0 7657E 00	61 5	234 9
9	0 6969E 01	0 1001E 01	0 7848E+01	0 8476E+00	38 8	278 2
10	0 5777E 01	0 2474E 01	0 6084E 01	0 7982E 00	83 2	293 2
11	0 3008E 01	0 8258E 01	0 3798E 01	0 5750E 00	-9 6	306.6
12	0-1992E 01	0 1935E+01	0 2791E 01	0 4456E 00	1 9	314 5

Fig 2.9 Example of computer print out. (The data are dra in Fig 3 1.A, see 14.5 kHz record). Key Row 1 Record 21 30 db (corresponds to 85 db SPL), 14.50 kHz. Column 1 electrode numbers 1 to 12, 2 sine (electrode output); 3 cosine (electrode output)' 4 CM amplitude in μV rms 5 $\log_{10}$ CM amplitude; 6, CM phase referred to phase value of electrode 1, 7 CM phase measured against stimulus signal.

all twelve electrode outputs were recovered in this way Then the computer data were stored on magnetic tape.

This technique allowed the rapid collection of data. During a session of three to four hours the responses due to about 80 different stimuli could be measured. In terms of the previously employed techniques this meant taking 80 × 12 × 2 = 1 920 different accurate amplitude and phase readings—quite an impossible task even if there had not been any noise.

After the data collecting phase sinusoidal curves were fitted to the data stored on the magnetic tape and subsequently the computer printed out amplitude and phase values. (Here also the phase difference was measured between the reference signal (stimulus) and the output signal.) An example of such a print-out is given in Fig. 2.9

Since the cables leading from the laboratory to the computer room were quite long there was cross-talk from the reference channel to the signal channel. The 'ghost signal' in the signal channel due to cross-talk had a magnitude corresponding to a CM amplitude of 0.05 to 0.5 μV rms. In cases where the CM had a value in the region of 2 to 5 μV rms the ghost signal was measured separately and later subtracted from the compound signal. The ghost signal was determined by measuring the response in the idle signal channel, i.e. with no CM signal applied.

DISCUSSION OF TECHNIQUE

It is expedient to discuss at this point some questions relevant to the application of the multi-electrode technique. There are basically two aspects which need consideration. The one aspect is concerned with the question whether the mechanical performance of the cochlear system is disturbed due to the slit opening and the insertion of the probe into scala tympani. The other aspect relates to the problem whether the mechanical presence of the array in the scala distorts the potential field set up by hair-cell activity

According to Johnstone & Boyle (1967) the fluid level in scala tympani has negligible influence on the mechanical response of the basal part of the basilar membrane thus there was no difference in their results whether the perilymph was present or absent in the basal scala tympani (provided the basilar membrane was not dried out. Johnstone, 1969) This is consistent with von Békésy's observations (1960 *a*, p. 439) on dimensional models where the emptying of 'scala tympani' did not alter the position of the vibratory pattern manifested by the local stability of the circular eddy in scala vestibuli Further evidence for the negligible effect of slit-like openings in the cochlear wall on the excitation pattern comes from the experiments by Tasaki et al. (1952). These authors made a small opening in the

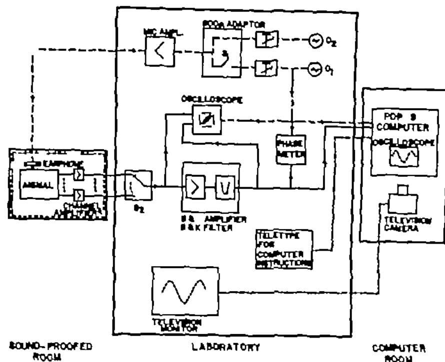


Fig 2.8 Block diagram of the ex perimental set-up. --- stimulus signal —— CM output signal —· reference signal (from stimulus); ——— Computer instructions and television link.

filtered CM response was read from the moni tor of the B & K amplifier The phase was determined in one of two ways. Feeding the output from the B & K amplifier into the oscilloscope together with the stimulus signal and displaying the Lissajous figures was one possibility In the other more accurate method a phase meter was employed. It measured the phase difference between the stimulus and the output signal The meter was built in this laboratory according to a circuit diagram published by Woodbury (1961) The accuracy of the meter was ±2 over the frequency range used in the experiments

For the graphical representation of the CM patterns the phase of the most basal electrode was taken as 'zero reference phase This was achieved simply by subtracting from all twelve phase readings the value measured for the most basal electrode.

An alternative method was adopted when it became necessary to measure the CM response at low SPLs (40 to 60 db) The output signals were too small and the noise in the recording system was too high for the previously described methods to work satisfactorily The recovery of the waveform of the output signal from noise was accomplished with the aid of a digital computer PDP 9 situated in the computer room. The scheme for the computer aided measurements was devised by my colleague, H. F Ross, who also wrote the computer programme The method was based on the technique known as signal averaging' The noisy signal was recurrently sampled at 50 successive points. The sampling points were so spaced in time as to cover one to two periods of the signal waveform Sampling was initiated by pulses derived from the zero-crossings of the reference signal which was provided by oscillator O_1 Corresponding samples from recurrent sweeps were added up thus reinforcing the periodic signal waveform at each repetition The signal waveform was displayed on the oscilloscope screen of the computer From there it was transmitted via a television link back to the laboratory In this way it was possible to observe the building up of the wave form of the output signal on the television screen during tonal stimulation The stimulus to the animal was turned off (by opening switch S_1) as soon as the waveform of the output showed a satisfactory sinusoidal shape of sufficient amplitude. For any given stimulus

21	30	14 50				
1	0 1483E 02	0 1705E 02	0 2354E 02	0 1372E 01	0 0	316 4
2	0.-1317E 02	0 1577E 02	0 2055E 02	0 1313E 01	3 7	320 1
3	0 7711E 01	0.-1509E 02	0.-1496E 02	0 1229E 01	16 4	332 8
4	0 3311E 01	0 1310E 02	0.-1251E 02	0.-1133E 01	29 4	345 8
5	0 6675E 00	0 1005E 02	0 1000E 02	0 1003E+01	7 5	323 9
6	0 1949E 01	0 2474E 01	0 3310E 01	0 5198E 00	55 7	260 7
7	0 3378E 01	0 5518E 00	0.-3415E 01	0 5334E 00	61 5	254 9
8	0 3430E+01	0.-1517E 01	0 5831E 01	0 7657E 00	38 2	278 2
9	0 6969E 01	0 1861E 01	0 7840E 01	0 6474E 00	83 2	233 2
10	0 5777E 01	0 2474E 01	0 6684E 01	0 7982E 00	9 6	306 8
11	-0 3068E 01	0 2258E 01	0 3756E 01	0 5758E 00	1 9	314 5
12	0 1998E+01	0 1955E 01	0 8791E 01	0 4450E 00		

Fig 2.9 Example of computer print out. (The data are drawn in Fig. 3.1.A, see 14.5 kHz record).
Key: Row *1*: Record 21, 30 db (corresponds to 85 db SPL), 14.50 kHz. Column *1*: electrode numbers 1 to 12, *2*: sine (electrode outputs); *3*: cosine (electrode output); *4*: CM amplitude in μV rms, *5*: $\log_{10}$ CM amplitude; *6*: CM phase referred to phase value of electrode 1, *7*: CM phase measured against stimulus signal.

all twelve electrode outputs were recovered in this way. Then the computer data were stored on magnetic tape.

This technique allowed the rapid collection of data. During a session of three to four hours the responses due to about 80 different stimuli could be measured. In terms of the previously employed techniques this meant taking $80 \times 12 \times 2 = 1\,920$ different accurate amplitude and phase readings—quite an impossible task even if there had not been any noise.

After the data collecting phase sinusoidal curves were fitted to the data stored on the magnetic tape and subsequently the computer printed out amplitude and phase values. (Here also the phase difference was measured between the reference signal (stimulus) and the output signal.) An example of such a print-out is given in Fig. 2.9.

Since the cables leading from the laboratory to the computer room were quite long there was cross-talk from the reference channel to the signal channel. The 'ghost signal' in the signal channel due to cross-talk had a magnitude corresponding to a CM amplitude of 0.05 to 0.5 μV rms. In cases where the CM had a value in the region of 2 to 5 μV rms the ghost signal was measured separately and later subtracted from the compound signal. The ghost signal was determined by measuring the response in the idle signal channel, i.e. with no CM signal applied.

DISCUSSION OF TECHNIQUE

It is expedient to discuss at this point some questions relevant to the application of the multi-electrode technique. There are basically two aspects which need consideration. The one aspect is concerned with the question whether the mechanical performance of the cochlear system is disturbed due to the slit opening and the insertion of the probe into scala tympani. The other aspect relates to the problem whether the mechanical presence of the array in the scala distorts the potential field set up by hair-cell activity.

According to Johnstone & Boyle (1967) the fluid level in scala tympani has negligible influence on the mechanical response of the basal part of the basilar membrane: thus there was no difference in their results whether the perilymph was present or absent in the basal scala tympani (provided the basilar membrane was not dried out. Johnstone, 1969). This is consistent with von Békésy's observations (1960 *a* p. 439) on dimensional models where the emptying of 'scala tympani' did not alter the position of the vibratory pattern manifested by the local stability of the circular eddy in 'scala vestibuli'. Further evidence for the negligible effect of slit-like openings in the cochlear wall on the excitation pattern comes from the experiments by Tasaki et al. (1952). These authors made a small opening in the

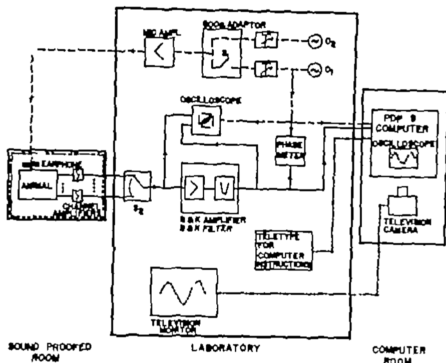


Fig 2.8 Block diagram of the experimental set up. --- stimulus signal —— CM output signal, — reference signal (from stimulus) -·-·- Computer instructions and television link.

filtered CM response was read from the monitor of the B & K amplifier The phase was determined in one of two ways Feeding the output from the B & K amplifier into the oscilloscope together with the stimulus signal and displaying the Lissajous figures was one possibility In the other more accurate method a phase meter was employed It measured the phase difference between the stimulus and the output signal. The meter was built in this laboratory according to a circuit diagram published by Woodbury (1961) The accuracy of the meter was ±2 over the frequency range used in the experiments.

For the graphical representation of the CM patterns the phase of the most basal electrode was taken as 'zero reference phase This was achieved simply by subtracting from all twelve phase readings the value measured for the most basal electrode

An alternative method was adopted when it became necessary to measure the CM response at low SPLs (40 to 60 db) The output signals were too small and the noise in the recording system was too high for the previously described methods to work satisfactorily The recovery of the waveform of the output signal from noise was accomplished with the aid of a digital computer PDP-9 situated in the computer room The scheme for the computer aided measurements was devised by my colleague, H. F Ross, who also wrote the computer programme The method was based on the technique known as 'signal averaging The noisy signal was recurrently sampled at 50 successive points. The sampling points were so spaced in time as to cover one to two periods of the signal waveform Sampling was initiated by pulses derived from the zero-crossings of the reference signal which was provided by oscillator O_1 Corresponding samples from recurrent sweeps were added up thus reinforcing the periodic signal waveform at each repetition The signal waveform was displayed on the oscilloscope screen of the computer From there it was transmitted via a television link back to the laboratory In this way it was possible to observe the building up of the waveform of the output signal on the television screen during tonal stimulation The stimulus to the animal was turned off (by opening switch S_1) as soon as the waveform of the output showed a satisfactory sinusoidal shape of sufficient amplitude For any given stimulus

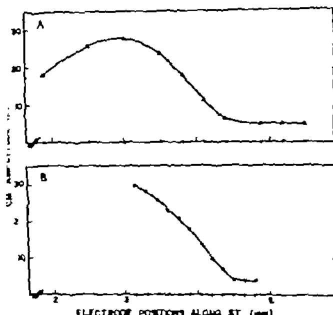


Fig. 2.10 CM amplitude pattern at 1 kHz, 90 db SPL measured in two different guinea pigs. (Electrode position along scala tympani, ST is given.)

(A) Pattern obtained with the single electrode technique (ten different electrodes).

(B) Pattern obtained with the 'multi-electrode' technique. The two different techniques gave the same results over the region studied.

a distortion of the eddy in 'scala tympani' however the eddy in 'scala vestibuli' remained unchanged. This effect seemed to be related to the narrowing of 'scala tympani' due to the presence of the object and von Békésy concluded 'that nature had made the depth of the duct as small as possible without going so far as to cause a spreading out of the vibratory pattern along the cochlear partition'. Since in our experiments there was sufficient free space between the actual array and the borders of the slit one would assume that the array did not effectively narrow the canal.

The other major question which needs consideration is concerned with the influence the mechanical presence of the array might have on the potential field in the scala. As shown in Fig. 2.10 both techniques employed in the study with their very different electrode arrangements gave the same results, hence it seems very unlikely that the array probe significantly distorts the potential field. In Fig. 2.11 A. the results from another test experiment are displayed. The CM potentials were measured for the same stimulus at two different depths of insertion of the probe into scala tympani. Thus the length of the array tip inserted in the scala was different for the two measurements. There are only very slight differences between the patterns acquired from different depths and we may assume that these differences are due to the conductive properties of the inner ear rather than the different lengths with which the array projects into the scala. Yet another piece of evidence is illustrated in Fig. 2.11 B. Here the CM pattern of an 8 kHz tone was registered. (The animal was exposed previously to a traumatic tone of 13 kHz; this explains the peculiar shape of the pattern. See Section 3 Tonal Overstimulation.) This pattern was chosen for the test experiment because there was a large phase shift between electrodes 5 and 6. The array was translated by a small amount in the longitudinal direction and the CM response to the same stimulus was measured again. As shown in the illustration we obtained essentially the same pattern, displaced however in the longitudinal direction. The large phase shift occurs now between electrodes 3 and 4. This is convincing evidence that the electrode probe itself had practically no influence on the results and can be regarded as a passive sensing device meeting the purpose for which it was intended.

wall of scala vestibuli in the second turn of the guinea pig's cochlea and they monitored the CM response in the third turn before and after the manufacture of the hole. Due to the hole the CM response dropped only by an amount of 3 to 4 db. (Tasaki et al. came to the very interesting conclusion that a considerable portion of the acoustic energy must be transmitted to the upper parts of the cochlea by means of longitudinal coupling in the membrane. This is important since mathematical models of cochlear hydrodynamics usually neglect coupling forces between the transverse elements of the basilar membrane (see Zwislocki, 1953).) It is difficult to judge whether the hole produced a complete release of the pressure wave in the fluid column of scala vestibuli and therefore prevented energy transport via the fluid medium to the regions apical to the hole. If this was the case the conclusion of Tasaki et al. would be necessary. Despite the fact that the hole had an area almost as large as the canal cross section it would probably be more realistic to visualize the effect of the hole as that of a 'hydraulic filter' with frequency dependent characteristics. On the basis of this assumption one would predict the occurrence of a large attenuation of the apical signal in cases where the wavelength of the pressure wave approaches the dimensions of the hole, i.e. at high frequencies where the wavelength is sufficiently short. Tasaki et al. reported that 'for all the frequencies employed the drop in potential in the third turn was the same. However, in the case of a hole (width 200 μ) connecting the basal scala vestibuli with the scala tympani of the second turn they recorded an equal change of the apical potential for frequencies below 800 Hz' this we may assume could mean that at higher frequencies apical potentials were relatively more affected. The problem of which medium—the membrane or the fluid—is mainly responsible for the energy transport is extremely difficult to solve with experiments like those of Tasaki et al. Not only can an opening influence the hydrodynamics of the system but it can also influence the recording situation: due to the outflow of perilymph from the opening there exists the possibility of electrical cross-connections between channels via the electrode holes. Clearly, the necessity to keep these two effects separate seriously narrows the range of possible spatial configurations of electrode positions and opening positions.)

Evidence for the negligible influence of the slit in the wall of the basal scala tympani on the functioning of the cochlea is provided by the present study. Fig. 2.10 shows the CM amplitude patterns obtained from two different guinea pigs for the same stimulus parameters. The one curve was measured with the single electrode technique, i.e. ten electrodes were essentially sealed into the holes in the wall of scala tympani. The other curve was recorded with the multi-electrode technique, i.e. the electrode array was inserted through the slit into scala tympani. It can be seen that both techniques produced the same result.

The weight of the evidence mentioned so far seems to support the view that the effect of the opening in the basal part of scala tympani can be neglected. However, there is a statement in the literature (von Békésy & Rosenblith, 1951, p. 1105; von Békésy 1960 *b*, p. 92) that a circular eddy occurs at any opening in the cochlear wall under strong tonal stimulation. It is stated further that covering the opening with a glass plate and immersing the whole preparation in fluid reduces the eddy to a great extent. In our experiments a considerable amount of perilymph was allowed to collect above the slit opening. Whether this fluid column above scala tympani had an effect similar to that of the measures taken by von Békésy is, of course, impossible to decide on the basis of CM recordings.

A further point concerns the fact that the array tip was inserted into scala tympani so that a mechanical object was present in the tympanic channel. This situation is similar to some extent to the model study of von Békésy (1960 *a*, p. 437) where a 'small object was placed in scala tympani'. Von Békésy observed

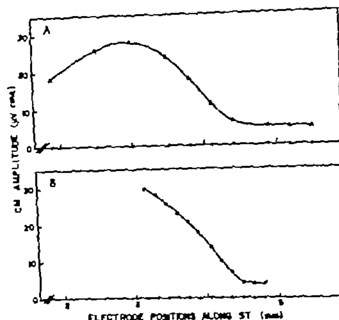


Fig. 2.10 CM amplitude pattern at 12 kHz, 90 db SPL, measured in two different guinea pigs. (Electrode position along scala tympani, ST is given.)

(A) Pattern obtained with the 'single electrode technique (two different electrodes).

(B) Pattern obtained with the 'multi-electrode technique. The two different techniques gave the same results over the region studied.

a distortion of the eddy in scala tympani' however the eddy in 'scala vestibuli remained unchanged. This effect seemed to be related to the narrowing of 'scala tympani' due to the presence of the object and von Békésy concluded 'that nature had made the depth of the duct as small as possible without going so far as to cause a spreading out of the vibratory pattern along the cochlear partition Since in our experiments there was sufficient free space between the actual array and the borders of the slit one would assume that the array did not effectively narrow the canal.

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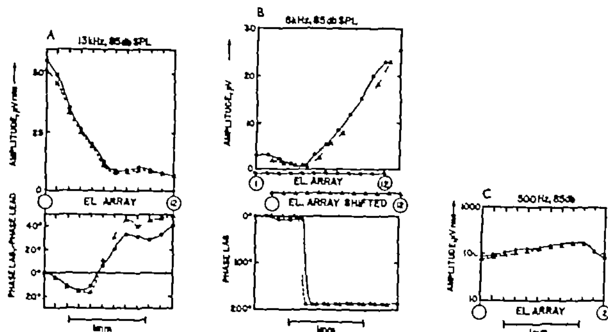


Fig. 2.11 (A) CM amplitude and phase pattern at 13 kHz, 85 db SPL. The array was inserted to a depth of 400 μ (●—●) and 300 μ (▲—▲) into scala tympani. There is very little difference between the CM patterns measured at different depths.

(B) CM amplitude and phase pattern at 8 kHz, 85 db SPL (after overstimulation with a tone of 13 kHz, 130 db for 3 min). The electrode array was translated in the apical direction by approximately 100 μ and essentially the same CM pattern was obtained. The large phase shift between electrodes 5 and 6 occurred after the translation between electrodes 3 and 4. ●—● Pattern before translation ▲—▲, pattern after translation.

(C) CM amplitude patterns obtained from two different guinea pigs for the same stimulus (500 Hz, 85 db SPL). The two curves are almost identical. ●—● first guinea pig. ▲—▲ second guinea pig. In (A), (B), (C), electrodes are numbered from 1 to 12 beginning with the most basal electrode.

Additional support for the validity of the CM recordings taken with the electrode array derives from the fact that each of the twelve electrode outputs had exactly the same properties as the CM recorded in the normal way by other workers with only one or two electrodes in the basal turn. This concerns the magnitude of the potentials and the intensity function. It suggests that the slit technique does not disturb the system more than the traditional technique with only a few small holes.

Theoretically speaking the multi-electrode technique cannot itself provide the answer to the question whether it causes disturbance in the cochlear system since such an answer requires the knowledge of the CM distribution in the completely undisturbed cochlea for comparison. The evidence presented above strongly suggests a negligible interference of the technique with the functions of the cochlea, but even if there was interference the technique would still be useful provided the interference was stable from experiment to experiment. The question of reproducibility of results was one of major concern in this study since any uncontrolled variability in the CM patterns would make the quantitative investigation of the spatial filter effect completely impossible. The great pains taken both with the surgery and the equipment to set up the experiment proved worth while in the long run. In Fig. 2.11 C two CM amplitude patterns from different guinea pigs are shown for the same stimulus parameters. It can be seen that the curves are almost identical. Thus experiments yielded reproducible data.

3 Results

The hypothesis to be tested by the experiments describes the cochlear *microphonic distribution* along the cochlear ducts as the spatially filtered version of the hair-cell activity along the basilar membrane.

$$CM(x) = \int_{-\infty}^{\infty} H(z)\, W(x-z)\, dz = H(x) * W(x) \quad (1)$$

Equation (1) shows the connection between $H(x)$ and $CM(x)$ for any arbitrary auditory stimulus. In our experiments sinusoidal stimuli were used and we may adopt the trigonometric or the alternative exponential notation to specify $H(x)$.

Sinusoidal stimulation of the inner ear causes travelling wave motion along the cochlear partition. The mechanical excursions of the partition activate the hair-cell generators. *We may represent the electrical output of any* such unit generator situated at a point x along the basilar membrane in terms of a Fourier series. For an external stimulus of angular frequency ω this series takes the form.

$$h(x_a, t) = A_0(x_a) + \sum_1^{\infty} A_n(x_a) \cos(n\omega t - B_n(x_a)) \quad (2a)$$

The expression (2 *a*) indicates that the electrical output at x may contain harmonic components due to nonlinearities in the transducer system. Since the space variable x assumes all *values x along the basilar membrane* $h(x, t)$ denotes the spatial distribution of the electrical output along the membrane:

$$h(x, t) = A_0(x) + \sum_1^{\infty} A_n(x) \cos(n\omega t - B_n(x)) \quad (2b)$$

or when written in exponential form

$$h(x, t) = A_0(x) + \sum_1^{\infty} \mathrm{Re}[A_n(x)\, e^{-jB_n(x)}\, e^{jn\omega t}] \quad (2c)$$

We obtain the instantaneous distribution of electrical *potentials along the cochlear scalae* $cm(x, t)$ by spatially filtering $h(x, t)$

$$cm(x, t) = \int_{-\infty}^{\infty} [A_0(z) + \sum_1^{\infty} \mathrm{Re} \times (A_n(z)\, e^{-jB_n(z)}\, e^{jn\omega t})]\, W(x-z)\, dz \quad (3a)$$

The time-dependent factors in (3 *a*) are unaffected by the spatial integration and we can write:

$$cm(x, t) = \int_{-\infty}^{\infty} A_0(z)\, W(x-z)\, dz + \sum_1^{\infty} \mathrm{Re} \times \left[e^{jn\omega t} \int_{-\infty}^{\infty} A_n(z)\, e^{-jB_n(z)}\, W(x-z)\, dz\right]$$

with $\int_{-\infty}^{\infty} A_0(z)\, W(x-z)\, dz = Q_0(x)$

$$\int_{-\infty}^{\infty} A_n(z)\, e^{-jB_n(z)}\, W(x-z)\, dz = Q_n(x)\, e^{-jP_n(x)}$$

It follows

$$cm(x, t) = Q_0(x) + \sum_1^{\infty} \mathrm{Re}[Q_n(x)\, e^{-jP_n(x)}\, e^{jn\omega t}]$$

$$cm(x, t) = Q_0(x) + \sum_1^{\infty} Q_n(x) \cos(n\omega t - P_n(x)) \quad (3b)$$

With a set *of sensing* electrodes placed along the cochlear duct the potential distribution $cm(x, t)$ can be recorded. By extracting the fundamental frequency of the electrode outputs we obtain the distribution of the fundamental *component.*

$$cm(x, t) = Q_1(x) \cos(\omega t - P_1(x)) \quad (4)$$

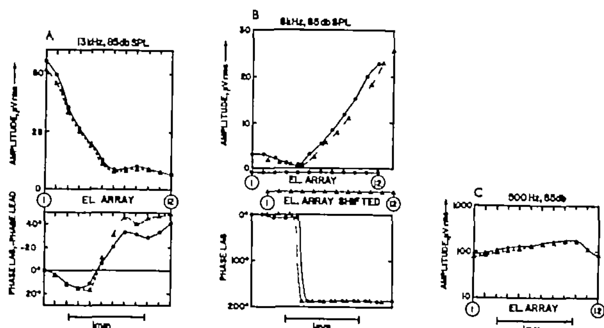


Fig 2 11 (A) CM amplitude and phase pattern at 13 kHz, 85 db SPL. The array was inserted to a depth of 400 μ (●—●) and 300 μ (▲—▲) into scala tympani. There is very little difference between the CM patterns measured at different depths.

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(C) CM amplitude patterns obtained from two different guinea pigs for the same stimulus (500 Hz, 85 db SPL). The two curves are almost identical ●—● first guinea pig. ▲—▲ second guinea pig. In (*A*), (*B*), (*C*) electrodes are numbered from 1 to 12 beginning with the most basal electrode.

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Equation (1) shows the connection between $H(x)$ and $CM(x)$ for any arbitrary auditory stimulus. In our experiments sinusoidal stimuli were used *and we may adopt* the trigonometric or the alternative exponential notation to specify $H(x)$.

Sinusoidal stimulation of the inner ear causes travelling wave motion along the cochlear partition. The mechanical excursions of *the partition activate the hair-cell generators.* We may represent the electrical output of any such unit generator situated at a point x_a along the basilar membrane in terms *of a* Fourier series. For an external stimulus of angular frequency ω this series takes the form.

$$h(x_a, t) = A_0(x_a) + \sum_{1}^{\infty} A_n(x_a) \cos(n\omega t - B_n(x_a)) \quad (2a)$$

The expression (2 *a*) indicates that the electrical output at x_a may contain harmonic components due to nonlinearities in the transducer system. Since the space variable x assumes all values x_a along the basilar membrane $h(x, t)$ denotes the spatial distribution of the electrical output along the membrane:

$$h(x, t) = A_0(x) + \sum_{1}^{\infty} A_n(x) \cos(n\omega t - B_n(x)) \quad (2b)$$

or when written in exponential form

$$h(x, t) = A_0(x) + \sum_{1}^{\infty} \mathrm{Re}[A_n(x)\, e^{-jB_n(x)}\, e^{jn\omega t}] \quad (2c)$$

We obtain the instantaneous distribution of electrical potentials along the cochlear scalae $cm(x, t)$ by spatially filtering $h(x, t)$:

$$cm(x, t) = \int_{-\infty}^{\infty} [A_0(z) + \sum \mathrm{Re}$$

$$\times (A_n(z)\, e^{-jB_n(z)}\, e^{jn\omega t})]\, W(x-z)\, dz \quad (3a)$$

The time-dependent factors in (3 *a*) are unaffected by the spatial integration and we can write:

$$cm(x, t) = \int_{-\infty}^{\infty} A_0(z)\, W(x-z)\, dz + \sum_{1}^{\infty} \mathrm{Re}$$

$$\times \left[e^{jn\omega t} \int_{-\infty}^{\infty} A_n(z)\, e^{-jB_n(z)}\, W(x-z)\, dz\right]$$

with

$$\int_{-\infty}^{\infty} A_0(z)\, W(x-z)\, dz = Q_0(x)$$

$$\int_{-\infty}^{\infty} A_n(z)\, e^{-jB_n(z)}\, W(x-z)\, dz = Q_n(x)\, e^{-jP_n(x)}$$

it follows

$$cm(x, t) = Q_0(x) + \sum_{1}^{\infty} \mathrm{Re}[Q_n(x)\, e^{-jP_n(x)}\, e^{jn\omega t}]$$

$$cm(x, t) = Q_0(x) + \sum_{1}^{\infty} Q_n(x) \cos(n\omega t - P_n(x)) \quad (3b)$$

With a set of sensing electrodes placed along the cochlear duct the potential distribution $cm(x, t)$ can be recorded. By extracting the fundamental frequency of the electrode outputs we obtain the distribution of the fundamental component.

$$cm(x, t) = Q_1(x) \cos(\omega t - P_1(x)) \quad (4)$$

The amplitude distribution $Q_1(x)$ and the phase distribution $P_1(x)$ *define the spatial distribution* of the fundamental component of the cochlear microphonic. Both parameter distributions can be determined experimentally by measuring the amplitude and phase values of all electrode outputs.

Since we are only interested in the amplitude and phase distributions it is preferable to use the exponential instead of the trigonometric notation. It is permissible to omit the time variable because it does not enter calculations. It therefore suffices to write

$$\mathrm{CM}_1(x) = \int_{-\infty}^{\infty} H_1(x)\, W(x-z)\, dz = H_1(x) * W(x) \qquad (5)$$

$$\mathrm{CM}_1(x) = Q_1(x)\, e^{jP_1(x)} \qquad (5a)$$

$$H_1(x) = A_1(x)\, e^{j\varphi_1(x)} \qquad (5b)$$

The expressions in (5 a) and 5 b) show the essential parameter distributions involved in the spatial filtering operation. The amplitude and phase distributions of the cochlear microphonic are related to the amplitude and phase distributions of the hair-cell outputs according to equation (5). Multiplication of equation (5) with a constant reference phase factor $\exp(jP)$ has no influence on the spatial integration. It is therefore permissible to refer the phase readings of the electrode outputs to any arbitrary phase value. In the following diagrams the output phases are always referred to the phase value of the most basal electrode. Since in our experiments we exclusively considered the fundamental component of the cochlear microphonic we may simplify the notation in the text. Thus, with $H(x)$ we mean the spatial distribution of the fundamental component of the hair-cell outputs in response to sinusoidal stimuli. $\mathrm{CM}(x)$ or $\mathrm{CM}(x_0)$ denotes the longitudinal distribution of the fundamental component of the cochlear microphonic in response to sinusoidal stimuli.

All CM patterns displayed in this section were obtained with the multi-electrode array. The most basal electrode of the array was always located in the region of 3 to 3.5 mm, *measuring* from the basal end of the basilar membrane. In the graphs the space coordinate is indicated by the electrode positions (see Fig. 3 1 B). It is expedient for the discussion of the CM patterns to express the space coordinate in terms of electrode numbers. Thus the electrodes of the array are numbered from 1 to 12 beginning with the most basal electrode.

A. THE DESCRIPTION OF DATA

CM amplitude and phase patterns

Experiments were carried out by measuring the amplitude and phase distribution of the CM along the electrode array for tonal stimuli of different frequencies. In all experiments we obtained response patterns with the same basic properties, all characteristic features of the distributions being reproducible in different guinea pigs.

Fig. 3 1 A shows a typical example of a set of CM patterns from one guinea pig: the patterns were measured at frequencies from 500 Hz to 15.5 kHz and a constant SPL of 85 db. The low frequency (500 Hz) record can be used as a control of the preparation and the uniformity of the sensing electrodes. At this frequency all individual electrodes composing the array are essentially equidistant from the maximum excitation which occurs in the apical portion of the cochlea. The wave length in the basal part of the excitation pattern is very long compared to the array width and we may therefore expect very little phase variation along the array. This expectation is confirmed by the 500 Hz record which shows negligible phase change between electrodes. Due to the long wavelength of the excitation pattern there is practically no phase cancellation in the region of the array and consequently the CM amplitude pattern has an appearance similar to that of the basal part of the envelope of the excitation pattern. Thus the amplitude in the 500 Hz pattern grows continuously in the apical direction. However at electrodes 11 and 12 the amplitude declines,

a feature observed in the majority of the preparations. The drop in potential is probably an artefact due to the strong curvature of the basilar membrane and the scala tympani in this region. Electrode tips are arranged in the array in a straight line which approximates to the curved course of the cochlear partition and cochlear canals over a short distance only. In terms of the array configuration the cochlear canals take a sharp bend away from the array in the region of electrodes 11 and 12. Thus 11 and 12 are the electrodes dislocated most from the ideal line following the longitudinal course of the membrane. It can be seen in the 500 Hz record that there is practically no alteration in the phase pattern at 11 and 12. This also indicates that the drop in amplitude at 11 and 12 is due to the placement of the array rather than phase cancellation. This conclusion results from the fact that in all high frequency records—where phase cancellation is manifest—amplitude variations are always accompanied by marked phase variations.

The goodness of a preparation may be judged on the basis of low frequency records because the expected patterns are simple and deviations from the normal response stand out clearly enough to allow rapid judgement. However, with the experience accumulated over many experiments it was possible to estimate the quality of preparations by considering the CM pattern at any frequency between 500 Hz and 15 kHz.

CM patterns take a more complicated form as the stimulus frequency is increased. The maximum of the excitation pattern along the membrane moves progressively towards the basal end of the cochlea with increasing frequency; the wavelengths of the excitation pattern become progressively shorter and this means that the phase lag gradient (phase change per unit length) along the partition increases with frequency. Closely neighbouring hair-cells are driven out of phase and consequently there will be negative interference—mutual cancellation of hair-cell outputs in the surrounding liquid spaces. The total number of hair-cells activated is probably smaller at high frequencies than at low ones because the excitation pattern is confined to a relatively shorter length of membrane. The general tendency of the CM amplitude to decrease when the stimulus frequency is raised can be read from Fig. 3.1.A. This tendency is probably due to the combined effect of the reduction in the number of activated hair-cells and the growing influence of phase cancellation with increasing frequency.

The CM amplitude distributions undergo characteristic changes with increasing stimulus frequency. Electrodes in the distant part of the array show relatively more drop in potential than do the proximal electrodes. The low level of the distant amplitudes is maintained when 15 kHz is exceeded and this observation indicates that the bulk of the excitation occurs in the region basal to this part of the array, that is at frequencies beyond 15 kHz. The most interesting feature, however, is the occurrence of a distinct amplitude minimum, well shown in the records at frequencies around 14 kHz.

The CM phase gradient along the array increases progressively with stimulus frequency (as shown in the records from 500 Hz to 12 kHz); thus at 12 kHz there is an overall phase difference between electrodes 1 and 12 of 196°. In the 13.5 kHz record this phase difference amounts to 258°; however, the shape of the CM phase distribution is clearly different from that in the 12 kHz record. The phase gradient is small in the proximal and distal parts of the array while it is large in the region of the amplitude minimum. The co-occurrence of an amplitude minimum and a large phase gradient is a characteristic property of CM patterns at high frequencies, in the present example at frequencies around 13.5 kHz.

At 13.75 kHz the phase gradient in the region of the amplitude minimum is even greater than at 13.5 kHz. The phase difference between electrodes 6 and 7 has a value of 143°. Clearly this is an enormous local varia-

The amplitude distribution $Q_1(x)$ and the phase distribution $P_1(x)$ define the spatial distribution of the fundamental component of the cochlear microphonic. Both parameter distributions can be determined experimentally by measuring the amplitude and phase values of all electrode outputs

Since we are only interested in the amplitude and phase distributions it is preferable to use the exponential instead of the trigonometric notation. It is permissible to omit the time variable because it does not enter calculations. It therefore suffices to write

$$\mathrm{CM}_1(x) = \int_{-\infty}^{\infty} H_1(x)\, W(x - \ )\, d = H_1(x) * W(x) \quad (5)$$

$$\mathrm{CM}_1(x) = Q_1(x)\, e^{jP_1(x)} \quad (5a)$$

$$H_1(x) = A_1(x)\, e^{jP_1(x)} \quad (5b)$$

The expressions in (5 *a*) and 5 *b*) show the essential parameter distributions involved in the spatial filtering operation The amplitude and phase distributions of the cochlear microphonic are related to the amplitude and phase distributions of the hair-cell outputs according to equation (5) Multiplication of equation (5) with a constant reference phase factor exp(*jP*) has no influence on the spatial integration It is therefore permissible to refer the phase readings of the electrode outputs to any arbitrary phase value In the following diagrams the output phases are always referred to the phase value of the most basal electrode Since in our experiments we exclusively considered the fundamental component of the cochlear microphonic we may simplify the notation in the text Thus, with $H(x)$ we mean the spatial distribution of the fundamental component of the hair-cell outputs in response to sinusoidal stimuli. CM(x) or CM(x_0) denotes the longitudinal distribution of the fundamental component of the cochlear microphonic in response to sinusoidal stimuli

All CM patterns displayed in this section were obtained with the multi-electrode array The most basal electrode of the array was always located in the region of 3 to 3.5 mm measuring from the basal end of the basilar membrane In the graphs the space coordinate is indicated by the electrode positions (see Fig 3 1 B) It is expedient for the discussion of the CM patterns to express the space coordinate in terms of electrode numbers. Thus the electrodes of the array are numbered from 1 to 12 beginning with the most basal electrode

A THE DESCRIPTION OF DATA

CM amplitude and phase patterns

Experiments were carried out by measuring the amplitude and phase distribution of the CM along the electrode array for tonal stimuli of different frequencies. In all experiments we obtained response patterns with the same basic properties, all characteristic features of the distributions being reproducible in different guinea pigs.

Fig 3 1 A shows a typical example of a set of CM patterns from one guinea pig the patterns were measured at frequencies from 500 Hz to 15.5 kHz and a constant SPL of 85 db Thu low frequency (500 Hz) record can be used as a control of the preparation and the uniformity of the sensing electrodes. At this frequency all individual electrodes composing the array are essentially equidistant from the maximum excitation which occurs in the apical portion of the cochlea. The wave length in the basal part of the excitation pattern is very long compared to the array width and we may therefore expect very little phase variation along the array Thus expectation is confirmed by the 500 Hz record which shows negligible phase change between electrodes. Due to the long wavelength of the excitation pattern there is practically no phase cancellation in the region of the array and consequently the CM amplitude pattern has an appearance similar to that of the basal part of the envelope of the excitation pattern Thus the amplitude in the 500 Hz pattern grows continuously in the apical direction However at electrodes 11 and 12 the amplitude declines,

A

B

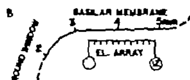


Fig. 3.1 (A) Typical example of a set of CM amplitude and phase patterns measured in one guinea pig. Note the co-occurrence of an amplitude minimum and a large phase gradient in the 13.5 kHz and 13.75 kHz record. CM phase patterns beyond 13.75 kHz show phase reversal.

(B) Position of electrode array (*EL. ARRAY*) in relation to the length coordinate of the basilar membrane. ——, amplitude; – – –, phase.

and phase data are considered in combined vector form. If the phase pattern is considered alone like in Fig. 3.1.A, the transition from the 13.75 kHz to the 14 kHz phase pattern appears to be an abrupt event, and therefore, quite irritating to the observer.

A simple model can be given to explain the transition from a trajectory with progressive

tion considering that the interelectrode distance is only 150 μ The appearance of the phase pattern changes abruptly when the stimulus frequency exceeds 13 75 kHz. Thus at 14 kHz the distant part of the array definitely leads the proximal part in phase This phase reversal in the CM pattern at high frequencies can only be observed by means of closely spaced electrodes. The technique of Tasaki et al. (1952) with only two recording points in the basal coil of the cochlea is completely inadequate for the study of the CM phase variation at high frequencies. The single electrode technique described in Section 2, with a minimum distance of 300 μ between electrodes was not good enough for the recording of complicated phase patterns like those displayed in the records from 14 kHz to 15.5 kHz. Comparison between the phase patterns at 15 kHz and at 15 5 kHz reveals a distinct shift of the 'zero-crossings maxima and minima of the 15.5 kHz phase patterns by about one electrode distance in the basal direction The overall phase variation along the array decreases noticeably from 14.5 kHz to 15.5 kHz.

We may summarize those features of the CM patterns which are most important for the demonstration of the spatial filter effect 1) Amplitude minima are always accompanied by large changes in phase between adjacent electrodes. 2) Phase reversal occurs at high stimulus frequencies and distant electrodes may lead proximal ones in phase

CM patterns in vector form

CM patterns represent complex functions of the real space variable x since they comprise two parameter distributions, those of amplitude and phase The two distributions may be plotted separately as shown in Fig 3 1.A, or they may be combined in a single 3-dimensional graph with the dimensions of the graph given by a set of complex planes arranged along the space coordinate x This is because an electrode output at x_n can be visualized as a complex number or a vector in the complex plane at x with the amplitude and phase value of the electrode output representing the vector length and vector phase angle respectively Thus it is easy to imagine the CM distribution as a line connecting the endpoints of the output vectors in the complex planes at the electrode positions 1 to 12. However for the purposes of the present discussion it is sufficient to consider the projection of this line into a single complex plane, say the plane at electrode 1 We may conveniently call the projected version of the CM distribution the vector representation or 'vector form It is constructed by simply plotting all twelve output vectors in the same plane and by joining the vector endpoints through a trajectory (For completeness of the definition it may be mentioned that this trajectory denotes the projection of the complex CM distribution into a single plane.)

In Fig. 3 2.A, B C the data from Fig 3 1 A are redrawn in vector form for the stimulus frequencies 13.5 13 75 and 14 kHz. Phase reversal occurs in this frequency region and the vectorial representation of data is best suited to illustrate this phenomenon At 13.5 kHz the CM pattern exhibits progressive phase lag along the array from 1 to 12. By measuring the phase lag in the clockwise direction we obtain for the 13.5 kHz record a trajectory which encircles the origin of the vector plane Clearly any trajectory characterised by a progressive phase lag encircles the origin With increasing stimulus frequency the trajectory approaches and eventually crosses the origin After crossing phase reversal within the vector bundle must occur because the trajectory no longer encircles the origin Thus in the 14 kHz record there is no encirclement of the origin and it can be seen that the phase reverses between electrodes 5 and 6 electrode 6 leading in phase electrode 5

Vector representation of the data discloses the occurrence of the phase reversal with increasing frequency as the result of a simple, continuous process. This is due to the fact that in this representation the CM amplitude

A

13.75 kHz 85 db
14.00 kHz 85 db
14.5 kHz 85 db
15 kHz 85 db
15.5 kHz 85 db
13.5 kHz 85 db

CM AMPLITUDE (μV rms)
CM PHASE (LAG LEAD)
EL. ARRAY
1 mm

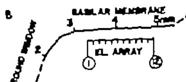


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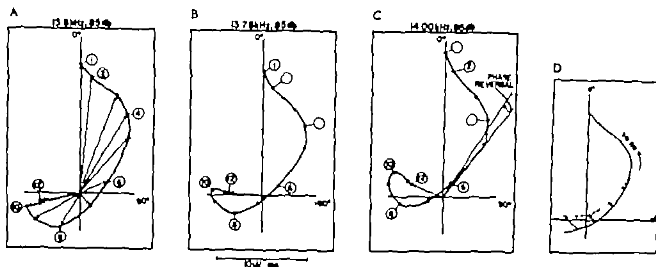


Fig 3.2 (A–C) Records (13.5 kHz, 13 75 kHz, 14 kHz) from Fig. 3 1.A redrawn in vector form. In (A) all output vectors are shown In (B) and (C) all vectors apart from 1 and 12 are omitted for clarity The trajectory connects all vector endpoints. Phase lag is counted in the clockwise direction The trajectory approaches with increasing frequency the origin of the vector plane note the concomitant increase in phase difference between 6 and 7 In (C) phase reversal occurs, 6 leading 5 in phase. In (A) and (B) the trajectory encircles the origin In (C) it does not.

(D) Artificially induced phase reversal by addition of small vector with large phase lag and amplitude which declines as the round window (*RW*) end of the array is approached

phase lag (13 75 kHz) into one with phase reversal (14 kHz) This is illustrated in the schematic drawing of Fig 3 2.D Let us add to all electrode outputs in a pattern like the one at 13 75 kHz a small vector with a large phase lag and a length which declines as the basal end of the array is approached The resulting trajectory has an appearance like the one at 14 kHz, i e it does not encircle the origin Something analogous to this vector addition may be expected to happen in reality As the stimulus frequency is increased from 13 75 to 14 kHz the wavelengths in the excitation pattern will shorten and a small group of distant hair-cells will be operating with slightly larger phase lag than before The influence of these distant hair-cell outputs is of course felt with an amplitude which declines towards the basal end of the array The model provides a qualitative explanation for the observed phase reversal and it suggests the far reaching influence of alterations in the outputs of subsets of hair-cell operators on the appearance of the whole CM pattern an influence postulated by the spatial filter hypothesis

A further point which deserves to be mentioned concerns the situation where the trajectory actually crosses the origin This situation was thoroughly explored in a preparation where one electrode of the array recorded very nearly zero amplitude in the frequency region of the phase reversal Thus this electrode output, when plotted on the trajectory very nearly coincided with the origin of the vector plane. The output of this electrode was displayed on the oscilloscope screen and a faint sinusoidal signal was just noticeable in the background noise It was interesting to observe that the output signal was extremely unstable shifting abruptly back and forth by about 180 on the oscilloscope screen All other electrode outputs however were perfectly stable This finding although it may seem strange at first glance can be interpreted quite plausibly Consideration of Fig 3 2 shows that the phase difference between the adjacent electrodes 6 and 7 grows continuously when the trajectory approaches the origin When the trajectory crosses the origin this phase difference becomes approximately 180 Thus a point in the immediate vicinity of the origin intermediate to 6 and 7 is framed by two vector bundles ap-

proximately 180° out of phase with each other This situation may be visualized as an unstable equilibrium, any small disturbance causing the small intermediate vector to shift to either of the two bundles.

B. THE ANALYSIS OF DATA

The spatial filter hypothesis is stated in the form of the convolution integral in equation (1):

$$CM(x) = H(x) * W(x) \qquad (1)$$

For the demonstration of the spatial filter effect the following mathematical procedure was adopted A certain hypothetical function $H(x)$ was assumed and subjected to the convolution in equation (1). The shape of $H(x)$ was chosen so as to resemble excitation patterns likely to exist in reality The resulting hypothetical $CM(x)$ distribution was examined for properties similar to the ones found in the experimental data. In the event that a hypothetical $CM(x)$ closely approximated to a given real CM pattern it was further assumed that the corresponding hypothetical $H(x)$ approximated to the real hair-cell output pattern along the basilar membrane.

However according to equation (1) $CM(x)$ depends on $H(x)$ and the weighting function $W(x)$ and consequently it is important to use in the mathematical analysis a function $W(x)$ based on experimental observations. Fortunately useful data about $W(x)$ are available in the literature.

The weighting function $W(x)$

The electrical attenuation properties of the cochlear ducts in the guinea pig were studied by four different investigators or groups of investigators (von Békésy 1960 *a*; Tasaki & Fernández 1952; Tasaki et al., 1952; Misrahy et al. 1958; Johnstone et al. 1966). All investigators agree that the longitudinal spread of current in the basal turn can be accurately described by a core conductor model. In the apical parts of the cochlea complication arises due to the close packing of turns and possible alternative paths of cross-conduction between turns. Since in our experiments data were collected in the basal turn we only need to consider conditions there.

Von Békésy thoroughly investigated the spread of signals along the basal part of the cochlear partition over a length of 9 mm beginning at a distance of 0.5 mm from the basal end of the membrane. He used a set of paired electrodes placed oppositely in scala tympani and scala vestibuli along the first turn. A test signal was injected across the partition at one pair of electrodes and the resulting attenuated signal was measured at the other sensing electrode pairs. The signal attenuation he obtained amounted to 6 db per mm. Moreover he observed that there was no remarkable change in the attenuation along the partition whether measured in the apical or basal direction. Thus the signal attenuation for a generator pair of electrodes situated at a position x along the partition is symmetrical with respect to x. Von Békésy employed signals with frequencies ranging from 100 Hz to 2 kHz and he could not find any dependence of the attenuation value on signal frequency This indicates that the signal attenuation along the partition is predominantly due to the resistive properties of tissues and fluids and that the capacitive properties of the structures have negligible influence Tasaki & Fernández applied a d.c. signal across the partition in the basal turn and they also measured a decay of signal from the site of application of 6 db per mm. Von Békésy's extensive study well established the core conductor type of the electrical attenuation along the partition and we can formulate mathematically the attenuation or weighting function:

$$W(x) = e^{-\lambda|x|} \quad \lambda = \frac{1}{\theta} \qquad (6)$$

x and θ in mm. θ denotes the length constant or decay constant of the longitudinal signal attenuation. In the core conductor model θ takes the form

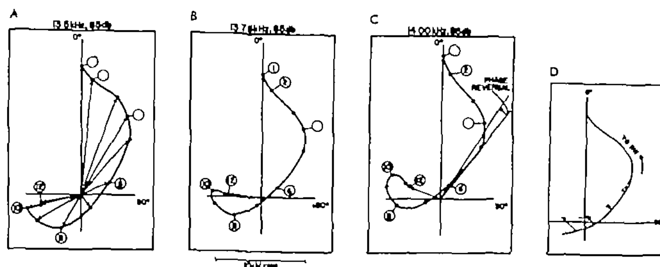


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cochlea. We have to adapt this pattern to high frequencies in order to make the distribution CM(x) resulting from the spatial filter operation comparable to our high frequency records. We achieved this adaption by compressing the von Békésy pattern into a shorter length of membrane. With the further assumption that hair-cell output is directly proportional to the local mechanical stimulus we obtained a hypothetical hair-cell distribution $H(x)$.

With the functions $H(x)$ and $W(x)$ (see equation (6)) given we can execute the spatial filter operation. The convolution integral

$$\mathrm{CM}(x_0) = \int_{-\infty}^{\infty} H(x) \exp(-0.69|x_0 - x|)\, dx$$

was solved by graphical integration.

Thus the above formula, continuous in x was substituted by

$$\mathrm{CM}(x_0) = \frac{m-n}{s} \sum_{i=0}^{s} H(x_i) \exp(-0.69|x_0 - x_i|) \quad (8)$$

where

$$x_i = n + i\frac{m-n}{s} \quad i = 0, 1, \ldots, s$$

and

$$x_i, x_0 \in [n, m]$$

In equation (8) the limits of the integration interval $[-\infty, \infty]$ are replaced by the finite boundaries n, m. This is permissible provided the bulk of the pattern $H(x)$ lies within the interval $[n, m]$. For the graphical integration the interval $[n, m]$ was subdivided in $s = 10$ equal intervals.

Each $H(x_i)$ in equation (8) is characterized by an amplitude and a phase value and can therefore be represented as a vector. Thus for each x_0 ($x_0 \in [n, m]$) CM(x_0) forms the vector sum of the contributions $H(x_i)$ weighted by the appropriate attenuation factor. In Fig. 3.3 all functions involved in spatial filtering are shown. The graphical integration yields the

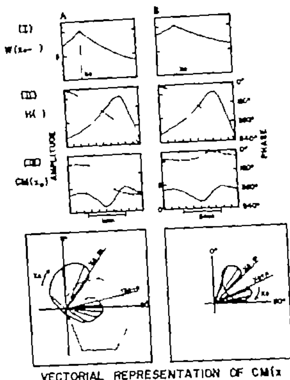


Fig. 3.3 Spatial Filtering of von Békésy pattern $H(x)$. In (A) $H(x)$ is compressed into interval $[n, m]$ = 2 mm. In (B), analogous to increase in frequency $[n, m]$ = 1 mm. All functions involved in spatial filtering are shown.

I. $W(x)$: weighting function, drawn for an arbitrary x_0. $W(x) = \exp(-0.69|x_0 - x|)$.

II. $H(x)$: hypothetical hair-cell output distribution of the von Békésy type.

III. CM(x_0): hypothetical CM distribution resulting from spatial filtering.

Bottom graph is the vector representation of CM(x_0). The convolution integral was solved by graphical integration, $[n, m]$ was subdivided into $s = 10$ equal intervals:

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As an example it is shown in the vector plane in (A) how CM(x_0) forms the vector sum of the weighted contributions $H(x_i)$ for $x_0 =$ ( ). In the 'lower frequency' example (A) the trajectory encircles the origin of the vector plane, in the 'higher frequency' example (B) it does not. Note co-occurrence of amplitude minimum and large phase gradient in (A,III). ——, amplitude; - - -, phase.

pattern CM(x_0) in vector form and it can be seen in the vector plot in (A) how the weighted vectors $H(x_i)$ add up to CM(x_0) for $x_0 = n$ (see

$$\theta = \frac{1}{\sqrt{dR\,dG}} \tag{7}$$

where dR signifies the longitudinal resistance per unit length of the fluid filled canals and dG signifies the longitudinal conductance per unit length of the cochlear partition. Tasaki et al measured the resistances involved and calculated (see equation (7) and (6)) the attenuation along the partition They obtained a value of 6 db per mm They measured an electrical impedance across the cochlear partition of 100 to 200 Ω per cm in the basal turn at a frequency of 500 Hz. At 6 kHz they found an impedance about 15% less. Since this variation in impedance with frequency is very small compared to the variation of impedance at a single frequency (500 Hz) we may consider the attenuation independent of frequency This implies, of course that λ in equation (6) is considered a real quantity that is, neglecting capacitive effects of intracochlear structures.

Data considered so far concerned the attenuation of signals across the cochlear partition The result, unanimously achieved, gives an attenuation of 6 db per mm i.e. a decay constant $\theta = 1.45$ mm ($\lambda = 0.69$) However it needs to be clarified whether this value of θ is a good approximation to the real situation where the signal is generated by the hair cells within the organ of Corti presumably across the reticular lamina and not as in the above test cases across the cochlear partition It is likely that a pair of electrodes, the one electrode situated in scala media and the other in scala tympani, mimics the real situation better than does a pair of electrodes situated across the whole cochlear partition Experiments to explore the attenuation along the basilar membrane have been done with electrodes inserted into scala media and scala tympani

Misrahy et al measured the resistances involved in this latter situation and they calculated (using equation (7)) for the basal turn a decay constant $\theta = 1.18$ mm for the signal spread along the basilar membrane However Johnstone et al measuring the spread of current pulses along the membrane with electrodes in scala media, obtained an average length constant $\theta = 2$ mm in the first turn The discrepancy between these results is probably due to the fact that these experiments are extremely difficult to execute requiring utmost surgical cleanliness, a requirement not easy to meet when handling sets of recording electrodes. Taking the arithmetic mean of the two values gives a mean length constant $\theta_m = 1.59$ mm a value not significantly different from the length constant along the whole cochlear partition Thus for the mathematical operations in this thesis we used von Békésy's value for the length constant corresponding to a signal decay of 6 db per mm.

The weighting function in equation (6) takes into account the longitudinal spread of signal along the cochlear scalae It does not take account of the signal variation across the scalae. In accordance with the core conductor model we may neglect as a first approximation the effect of the distance between electrode tips and the basilar membrane on the CM distribution In Fig 2.11 A the CM pattern for the same stimulus parameter is shown measured at two different depths of insertion of the electrode array into scala tympani thus the distance between electrode tips and the basilar membrane was slightly different in the two records. There are only minor differences between the two patterns and we may therefore regard the potential variation across the scala tympani negligible as a first approximation.

Spatial filtering of hypothetical patterns $H(x)$

It is expedient to begin the mathematical analysis of the spatial filter effect by subjecting a function $H(x)$ to spatial filtering which has the shape of the mechanical excitation curves measured by von Békésy The highest stimulus frequency at which von Békésy observed the complete mechanical excitation pattern along the membrane was 300 Hz the pattern extended from about 19 mm to 29 mm along the basilar membrane of the human

cochlea. We have to adapt this pattern to high frequencies in order to make the distribution CM(x) resulting from the spatial filter operation comparable to our high frequency records. We achieved this adaption by compressing the von Békésy pattern into a shorter length of membrane. With the further assumption that hair-cell output is directly proportional to the local mechanical stimulus we obtained a hypothetical hair-cell distribution $H(x)$

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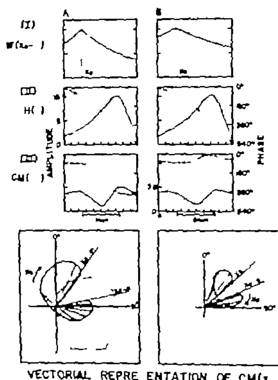


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where dR signifies the longitudinal resistance per unit length of the fluid filled canals and dG signifies the longitudinal conductance per unit length of the cochlear partition Tasaki et al. measured the resistances involved and calculated (see equation (7) and (6)) the attenuation along the partition They obtained a value of 6 db per mm They measured an electrical impedance across the cochlear partition of 100 to 200 Ω per cm in the basal turn at a frequency of 500 Hz. At 6 kHz they found an impedance about 15% less. Since this variation in impedance with frequency is very small compared to the variation of impedance at a single frequency (500 Hz) we may consider the attenuation independent of frequency This implies, of course, that λ in equation (6) is considered a real quantity that is, neglecting capacitive effects of intracochlear structures.

Data considered so far concerned the attenuation of signals across the cochlear partition The result unanimously achieved gives an attenuation of 6 db per mm i e a decay constant $\theta = 1.45$ mm ($\lambda = 0.69$) However it needs to be clarified whether this value of θ is a good approximation to the real situation where the signal is generated by the hair cells within the organ of Corti presumably across the reticular lamina and not as in the above test cases across the cochlear partition. It is likely that a pair of electrodes, the one electrode situated in scala media and the other in scala tympani mimics the real situation better than does a pair of electrodes situated across the whole cochlear partition. Experiments to explore the attenuation along the basilar membrane have been done with electrodes inserted into scala media and scala tympani

Misrahy et al. measured the resistances involved in this latter situation and they calculated (using equation (7)) for the basal turn a decay constant $\theta = 1.18$ mm for the signal spread along the basilar membrane However Johnstone et al. measuring the spread of current pulses along the membrane with electrodes in scala media, obtained an average length constant $\theta = 2$ mm in the first turn The discrepancy between these results is probably due to the fact that these experiments are extremely difficult to execute requiring utmost surgical cleanliness, a requirement not easy to meet when handling sets of recording electrodes. Taking the arithmetic mean of the two values gives a mean length constant $\theta_m = 1.59$ mm a value not significantly different from the length constant along the whole cochlear partition Thus for the mathematical operations in this thesis we used von Békésy's value for the length constant corresponding to a signal decay of 6 db per mm

The weighting function in equation (6) takes into account the longitudinal spread of signal along the cochlear scalae it does not take account of the signal variation across the scalae In accordance with the core conductor model we may neglect as a first approximation the effect of the distance between electrode tips and the basilar membrane on the CM distribution In Fig 2.11 A the CM pattern for the same stimulus parameter is shown measured at two different depths of insertion of the electrode array into scala tympani thus the distance between electrode tips and the basilar membrane was slightly different in the two records. There are only minor differences between the two patterns and we may therefore regard the potential variation across the scala tympani negligible as a first approximation

Spatial filtering of hypothetical patterns $H(x)$

It is expedient to begin the mathematical analysis of the spatial filter effect by subjecting a function $H(x)$ to spatial filtering which has the shape of the mechanical excitation curves measured by von Békésy The highest stimulus frequency at which von Békésy observed the complete mechanical excitation pattern along the membrane was 300 Hz, the pattern extended from about 19 mm to 29 mm along the basilar membrane of the human

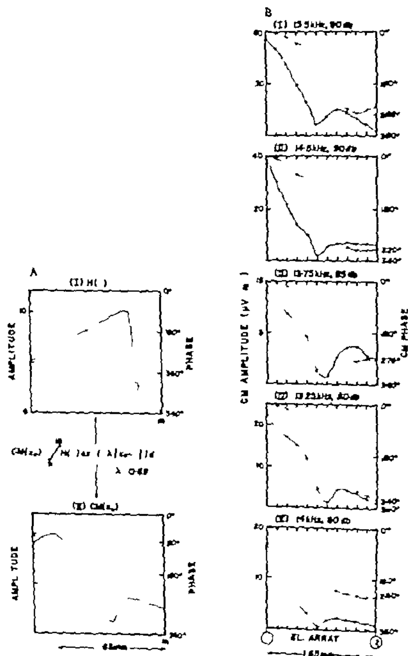


Fig. 3.5. (A.I–II) Hypothetical $H(x)$ and $CM(x_n)$ re-drawn from Fig. 3.4 B. Compare $CM(x_n)$ over the length 1.65 mm with experimental CM patterns in (B.I–V).

(B.I–V) Experimental CM amplitude and phase patterns obtained from five different guinea pigs. Stimulus parameters were essentially the same (stimulus frequency varied from 13.5 kHz to 14.5 kHz; SPL varied from 80 to 90 db). Note: Distinct amplitude minimum around 6 and 7; flat amplitude maximum around 8 and 9; large phase gradient in the region of amplitude minimum. On account of good quantitative agreement between $CM(x_n)$ (A.II) and experimental CM patterns (B.I–V) we assume $H(x)$ in (A.I) to be a close approximation—$H(x)^*$—to the real hair cell output patterns for the stimuli in (B.I–V), i.e. for stimulus frequencies between 13.5 and 14.5 kHz. ——, amplitude, - - - phase.

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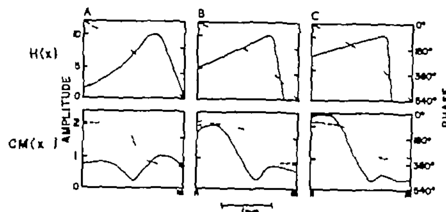


Fig. 3.4. Modification of 'von Békésy type' pattern $H(x)$. (A) $H(x)$ redrawn from Fig. 3.3.A. (B, C) Flattening the basal slope and steepening the apical slope of the envelope of $H(x)$ produces theoretical curves $CM(x_0)$ more similar to experimental CM patterns. —— amplitude ––– phase.

dashed line in vector diagram (A)). By plotting the amplitude and phase pattern of $CM(x_0)$ separately we obtain the hypothetical cochlear microphonic distribution $CM(x_0)$ in a fashion ready for comparison with experimental patterns.

In Fig. 3.3.A $H(x)$ was compressed into an interval length of 2 mm while in (B) analogous to an increase in frequency the interval length was only 1 mm. Let us call A the 'lower frequency' and B the 'higher frequency' example.

Practically all significant features of the resulting patterns $CM(x_0)$ are consistent with experimental findings. The amplitude of $CM(x_0)$ in the 'higher frequency' case is smaller than in the 'lower frequency' case; this is compatible with real patterns which showed an overall drop in amplitude with increasing stimulus frequency. The overall phase variation along the space coordinate in the 'higher frequency' example is very small, a feature well shown by the experimental patterns in Fig. 3.1.A at high stimulus frequencies. In the 'low frequency' example there is progressive phase lag along x_0, thus the trajectory in the vector representation encircles the origin of the vector plane. In the high frequency example no encirclement of the origin occurs and the phase pattern shows phase reversal, the more apical part of the pattern leading in phase the more basal part. The occurrence of phase reversal with increase in stimulus frequency was one of the main characteristics of experimental CM patterns. In the 'lower frequency' example we observe the co-occurrence of an amplitude minimum and a large phase gradient. This also was a main characteristic of experimental CM patterns.

Thus it is demonstrated that all salient features of the measured CM patterns can be qualitatively reproduced by spatially filtering an excitation pattern of the 'von Békésy type'. It should be noted that $CM(x_0)$ at a particular point x_0 does not reflect the hair-cell output at this point x_0; this can be clearly seen in Fig. 3.3. Thus the notion held by several investigators that an electrode records local hair-cell activity is proved incorrect.

Quantitative comparison between theoretical and experimental CM patterns

In the foregoing section we showed the excellent qualitative agreement between theoretical CM patterns deducible from the 'von Békésy type' function $H(x)$ and our own experimental data. Yet, closer inspection of the theoretical curves $CM(x_0)$ in Fig. 3.3 reveals that the quantitative agreement with measured patterns is rather poor; this applies especially to the shape of the amplitude pattern of $CM(x_0)$ which shows a large distant maximum.

We attempted to improve the quantitative fit between the theoretical and measured CM patterns by systematically modifying the shape of the hypothetical pattern $H(x)$. Since $H(x)$ consists of two parameter distributions, those of amplitude and phase, we can manipulate either or both of the two functions. Due to the lack

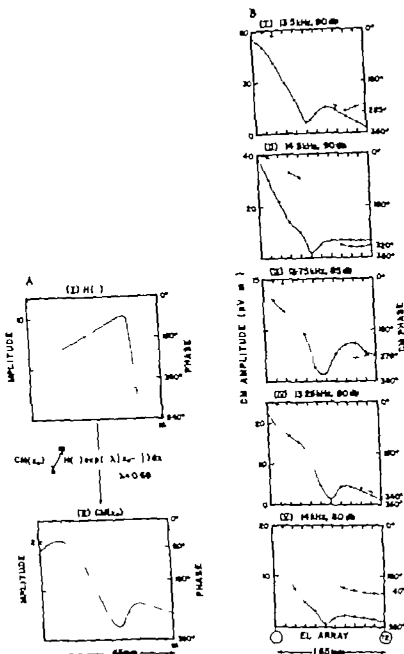


Fig. 3.5. (A.I, II) Hypothetical $H(x)$ and $CM(x_0)$ redrawn from Fig. 3.4 B. Compare $CM(x_0)$ over the length 1.65 mm with experimental CM patterns in (B.I–V).

(B.I–V) Experimental CM amplitude and phase patterns obtained from five different guinea pigs. Stimulus parameters were essentially the same (stimulus frequency varied from 13.5 kHz to 14.5 kHz, SPL varied from 80 to 90 db). Note: Distinct amplitude minimum around 6 and 7, flat amplitude maximum around 8 and 9, large phase gradient in the region of amplitude minimum. On account of good quantitative agreement between $CM(x_0)$ (A.II) and experimental CM patterns (B.I–V) we assume $H(\ )$ in (A.I) to be a close approximation—$H(x)^*$—to the real hair cell output patterns for the stimuli in (B.I–V), i.e. for stimulus frequencies between 13.5 and 14.5 kHz. ——, amplitude, – – phase.

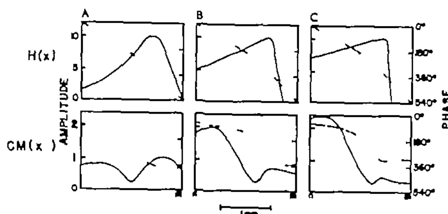


Fig 3 4 Modification of Békésy type pattern $H(x)$ $H(x)$ *redrawn from Fig* (B, C). Flattening the ba and steepening the apical the envelope of $H(x)$ pro theoretical curves $CM(x_0)$ similar to experimental C terns. —— amplitude - phase.

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"H(x)" and the frequency response curve

We obtained from the considerations outlined in the previous section a distribution $H(x)$" representing the approximate shape of the hair cell activity pattern along the membrane at stimulus frequencies from 13.5 kHz to 14.5 kHz. We are now in the position to compare this information about the excitation pattern with the data available from mechanical measurements. However $H(x)$ is a distribution along the space variable x while all the mechanical data are frequency response curves of points along the membrane, i.e. they are functions of the frequency variable. For the comparison between the two sets of data we have to deduce from the shape of $H(x)$" the corresponding frequency response curve. This deduction can be done provided we know the conversion formula relating the space variable x to the stimulus frequency f in the guinea pig's cochlea.

Greenwood (1961) formulated the connection between stimulus frequency and the location of the maximum displacement along the basilar membrane in the guinea pig. He used the expression.

$$f = A(10^{a[illegible]} - 1) \qquad (9a)$$

where f denotes stimulus frequency in Hz and [illegible] denotes the distance in mm of the maximum displacement from the apical end of the membrane. In this thesis we measured the spatial coordinate x from the basal end of the membrane. In the guinea pig the basilar membrane has a total length of 18.5 mm. Thus by using the substitution

$$r = 18.5 - x$$

we can write equation (9 a) in the form.

$$f = A(10^{[illegible]} - 1) \qquad (9b)$$

Greenwood determined the values ([illegible]) by fitting the function (9 a) to von Békésy's mechanical data measured in the upper turns of the cochlea. He obtained two sets of values, the one set giving rise to function G

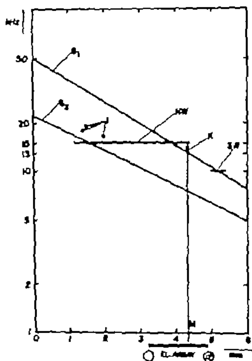


Fig. 3.7. Stimulus frequency on logarithmic scale plotted versus locus of maximum displacement along the guinea pig's basilar membrane. The space coordinate is measured from the basal end of the membrane, it is given in mm.
G and G Greenwood's functions.
SW Damage of organ of Corti after overstimulation with 10 kHz tone, observed by Smith & Wever (1949).
NW Region of swollen hair-cell nuclei after exposure to 15 kHz tone, observed by Neubert & Wüstenfeld (1955).
F Frequency of maximum displacement for points along the guinea pig's membrane, observed by Johnstone & Boyle (1967) and Johnstone (1969).
K. The position of the electrode array relative to the space coordinate is shown. M indicates the position of the envelope maximum of $H(x)$" according to Fig. 3.5. M falls into the region between electrodes 8 and 9. With M and the stimulus frequencies (13.5 kHz to 14.5 kHz) given for which $H(x)$" is considered an approximation to the hair-cell output distribution we can plot our results into the graph. They are marked K and they show good agreement with G

$$G_1 \quad f = 166.2(10^{[illegible]} - 1)$$

the other set leading to G_2,

$$G_2 \quad f = 253(10^{[illegible]} - 1)$$

Both functions G_1 and G_2 are shown in Fig. 3.7. The function G_2 fits the low frequency

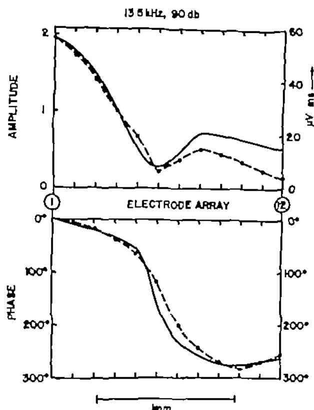


Fig 3.6 Hypothetical CM(x_0) from Fig. 3 4 B and Fig. 3.5.A.II drawn together with experimental CM pattern from Fig. 3.5.B I The relative amplitude for CM(x_0) is indicated on the left hand scale the amplitude for the experimental 13.5 kHz, 90 db pattern is indicated on the right hand scale in μV rms. Both amplitude and phase patterns show good quantitative agreement. —— hypothetical CM(x_0) - - - measured CM pattern at 13.5 kHz, 90 db.

of experimental data on the longitudinal phase variation in the mechanical excitation pattern at frequencies beyond 300 Hz we left the phase pattern of $H(x)$ unchanged. The amplitude envelope of $H(x)$ was modified and the corresponding CM(x_0) was calculated by graphical integration according to equation (8)

In Fig 3 4 the result of this procedure is shown Fig 3 4 A displays the 'von Békésy type pattern $H(x)$ used in Fig 3.3 A It can be seen that flattening the basal slope and steepening the apical slope of the envelope of $H(x)$ produces CM(x_0) patterns which approximate in shape very closely to experimental CM patterns. The CM(x_0) in Fig 3 4 B gives the closest fit and it is redrawn in Fig. 3.5 A together with experimental CM distributions.

It can be seen at once that there is very g agreement between the hypothetical CM in Fig. 3.5 A II and the measured CM patt in Fig 3 5 B I to V The experimental patt were obtained from five different guinea p The stimulus parameters in all five cases w essentially the same thus the stimulus quency varied only from 13.5 kHz to 14.5 k and the stimulus intensity varied from 80 90 db SPL. All experimental amplitude p terns exhibit a distinct minimum around el trodes 6 and 7 and a flat maximum arou electrodes 8 and 9 In the region of the am tude minimum all patterns show the char teristic large phase variation The overall ph change along the array amounts in the f cases to 285 320 276 350 240 resp tively For the comparison between the th retical and experimental CM patterns, we co sider the theoretical pattern over a length 1 65 mm equal to the width of the electr array The overall phase variation in t length interval of the theoretical curve has value of 280 Undoubtedly there is very go quantitative agreement between the theoreti and experimental curves.

In order to show the quantitative fit mo clearly the measured CM pattern of Fig 3.5 E was redrawn along with the theoretical patte of Fig. 3.5 A.II in the same diagram in Fi 3 6 The excellent agreement between t hypothetical and the measured CM patter well documented in Fig 3 6 suggests the fo lowing logical conclusion the hypothetical pa tern $H(x)$ from which the hypothetical CM(x in Fig 3 6 was deduced must approximate ve closely to the real hair-cell output patter which produced the measured CM distribu tion in Fig 3 6

Thus in the following discussion we shall a sume that the hair-cell output pattern at stimu lus frequencies from 13.5 kHz to 14.5 kHz ca be represented by the hypothetical patter "$H(x)$" given in Fig 3.5 A.I (Writing $H($ with inverted commas indicates that "$H(x)$" i considered to be an approximation to the rea curve)

"H(x)" and the frequency response curve

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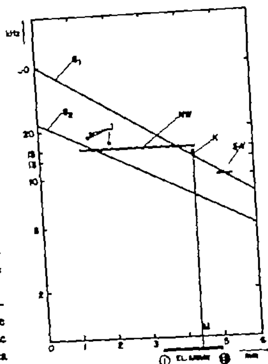


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Fig. 18. Detail (enlargement) of Fig. 17. 100 000.

for the enzyme if penetration of the substrate and of the lead salt is possible. Recent studies indicate that the enzymes are active inside the lysosomes (De Duve, 1970). The first sign of a pathological process is the occurrence of a positive enzyme reaction as well as incipient intracellular lysis. It is as if the lysosomes—for reasons still unknown—start growing and that the permeability of their membrane alters in a way so that the hydrolases can get into contact with the surrounding cytoplasm. At this stage electron microscopy shows small, round, empty vesicles exhibiting centrally a positive reaction with lead deposits surrounded by a small lytic zone. From then on the development will be very rapid. The lytic zones grow in size and number. In a few cases we have been able to visualize the exocytosis of the hydrolases, a large vesicle opening into the surrounding tissue where incipient destruction is taking place (Fig. 2). Without a doubt this process will, despite the exocytosis phenomenon, also destroy the cell itself. The destruction soon gives rise to swelling of the mitochondria which lose their crests. In this way the mitochondria may give a positive enzyme reaction, meaning that no further active oxidation enzymes are left (Figs. 17, 18). Vesicular degeneration of the cell will occur rapidly, the entire cytoplasm getting vesicular. The nucleus itself also undergoes lysis, and the result soon becomes a "ghost cell" (Figs. 4, 19). At this stage, it is difficult



Fig 19 Extensive cytoplasmatic lysis with clear-cut enzymatic activity 19 000

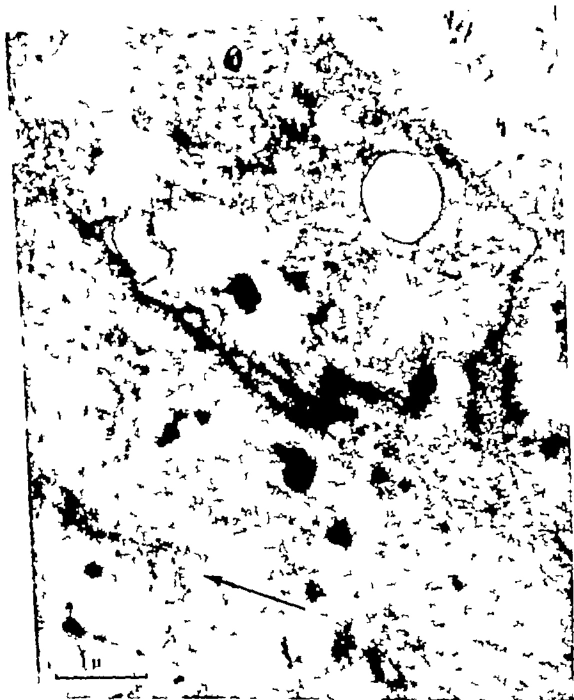


Fig. 70. Active enzymatic process around the cell lacuna and the canaliculi. 30 000



Fig 19 Extensive cytoplasmatic lysis with clear-cut enzymatic activity 19 000

activity which they designated *osteolysis* (or osteocytic resorption). This process involves, at the same time, rapid maturation of osteocytes and changes in the surrounding bony matrix, so that at this site there is a low concentration of mineral as well as of metachromatic organic material consisting of mucopolysaccharides staining with PAS. This is presumably approximately the same mechanism which we observed in the destructive phase of otosclerosis.

Recently it was demonstrated by Andersen et al. (1969), on the basis of studies on the growth of the otic capsule in the human foetus, that at an early stage of foetal life the cavities in the otic capsule are formed by histiocytes possessing the ability for chondrolysis.

The teory on osteoclastic resorption, then, cannot answer all the questions posed by recent studies.

During the past 10 years, the electron microscope has made it possible despite great technical difficulties to determine the sub-microscopic structure of the osteocytes. Especially Baud (1962, 1968) and Bélanger et al. (1963 1965) have supplied the morphological basis for investigating the physiological role played by these cells.

Several authors (Ruyter 1964 Manning & Butler 1965 Baud, 1968) have demonstrated the presence of lysosome-like bodies in the osteocytes by the histochemical demonstration of acid phosphatase activity in the osteocytes.

Other lysosomal hydrolytic enzymes capable of digesting proteins (Bélanger & Migiocovsky 1963 Woods & Nichols, 1965) as well as mucopolysaccharides (Schlager 1959 Gubisch & Schlager 1961) are also present and may ensure the attack upon organic bone matrix.

As far as bone resorption is concerned the possible role of lysosomes and, incidentally the value of acid phosphatase activity has been studied by Scott & Pease (1956), Gonzales & Karnovsky (1961), and Hancox & Boothroyd (1961) Woods & Nichols (1965) found some fractions of rat bones to be rich in collagenase activity Goldhaber (1961) demonstrated that resorption of bone was followed by degradation of bone collagen. One or several enzymes are released during the resorption of bone (Stern, 1965), and this or these enzymes have collagenolytic activity The studies of Kaufman et al. (1965) confirmed that active resorption of bone in tissue cultures is followed by a release of a collagenolytic factor capable of breaking down collagen fibrils at a neutral pH and 37 C. This process has been found to be increased by parathyroid extract (Walker et al., 1964) and heparin.

The disagreement between the osteoclastic resorption theory and the enzyme theory which we are advancing here—and which can explain our light microscopic as well as electron microscopic findings—is perhaps more apparent than real. It is known that when the osteoclasts act locally through their ruffled borders and absorb calcium hydroxyapatite crystals by pinocytosis, they may also exert an important acid phosphatase activity (Schajowicz & Cabrini, 1958 Burstone 1959 1962 Handelman et al., 1964), an activity which perhaps plays a role at a certain distance from the cell, although this has never been demonstrated.

The enzymatic lysosomal theory we are advancing is based upon morphological as well as experimental findings. Morphologically as previously mentioned, the *regular centrifugal* extension of large destruction foci (in the way of a "front") as well as the findings of small *foci "splashing* around can only be explained by an enzymatic process. The presence of cells showing in their cytoplasm a varying number of lysosomal, dense bodies, and which we have called histiocytes (although names are not the cardinal point) guarantee the enzymatic material needed for the bone resorption. The activity of the numerous hydrolytic enzymes found in the lysosomes explains—by reason of the protease, hyaluronidase, cathepsin, and acid phosphatase content—why destruction of the tissue may take place, although it is, as in this case, a dense bony tissue with abundant collagen. In addition, we have demonstrated the extension of cell destruction caused by changes in the permea-

to follow the localization of the enzyme, as it diffuses into the lacuna, the canaliculi, and the surrounding tissue. However despite advanced lysis, we have in a few cases been able to find occasional apparently intact lysosomes in the focus.

9 Discussion

In the present study we have tried to place otosclerosis research on a cellular basis Thereby we have attempted to throw some light upon the problem relating to the lytic phase of the otosclerotic process.

The course of the disease cannot be divided, clearly and simply into two successive phases, a destructive and a rebuilding one It runs a long course during which the changes do not automatically follow upon each other There is no doubt that quiet phases occur between two acute ones. In addition destructive and rebuilding phases may be observed at the same time in neighbouring foci However the destructive or lytic phase is always the first one, so that it is invariably present at the onset of the disease As pointed out in the section on the biochemistry of bone formation, it is presumably in this phase that the cellular mechanism is most difficult to understand

Claiming that it is a classical fact that osteoclasts alone are responsible for the destruction is presumably not entirely true No doubt osteoclasts play a decisive role in the resorption and remodelling of *normal* long bones On the other hand it is by no means certain that they play a role in all *abnormal* processes of bone resorption We have previously discussed the relative *absence of osteoclasts in active otosclerotic* foci To elucidate the occurrence of osteoclasts in the otosclerotic focus we studied 84 foci in 56 otosclerotic temporal bones in the light microscope. 30 of these foci proved to be highly active 32 of moderate activity and 22 inactive No osteoclasts were demonstrable in any specimen from foci that showed no or moderate activity (a total of 54) Of the 30 foci with high activity 6 were found to contain many osteoclasts, 8 scattered, and 3 occasional osteoclasts. Thirteen highly active foci were found to be entirely devoid of osteoclasts We have noticed also that the osteoclasts, when present, are almost invariably in the centre of the focus where brisk remodelling of newformed bony tissue takes place On the other hand we could not demonstrate osteoclasts in the marginal area of the focus where resorption of the original labyrinthine capsule takes place.

In addition, the precise and constant morphological electron microscopic findings in the extension zone of the active focus do not agree with the classical theory on osteoclastic resorption When actively engaged in a process of bone resorption, the osteoclasts generally make deep irregular encroachments into the bony tissue which is being destroyed Our regular finding of a distinct, well-defined "front" indicates that there can be no question of such a *destruction under these conditions Furthermore*, it is difficult to explain how microfoci also a constant finding, may be present in the tissue which is still normal and at some distance from the focus, if osteoclasts were the only cause of bone resorption in the otosclerotic focus

Osteoclasts need not be the only cause of bone resorption Various authors have suggested that the osteocytes may be capable of limited resorption of their lacunar walls (Heller Steinberg. *1951 Kind 1951) Especially after treat*ment with parathyroid extract (Heller Steinberg 1951 Jowsey 1960 Talmage 1962) osteocytes have been shown to take part in bone resorption However it was Bélanger and his coworkers (1963 1965) who described a specific form of bone resorption related to osteocytic

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Several authors (Ruyter 1964 Manning & Butler 1965 Baud, 1968) have demonstrated the presence of lysosome-like bodies in the osteocytes by the histochemical demonstration of acid phosphatase activity in the osteocytes.

Other lysosomal hydrolytic enzymes capable of digesting proteins (Bélanger & Migicovsky 1963 Woods & Nichols, 1965) as well as mucopolysaccharides (Schlager 1959 Gubetch & Schlager 1961) are also present and may ensure the attack upon organic bone matrix.

As far as bone resorption is concerned, the possible role of lysosomes and, incidentally the value of acid phosphatase activity has been studied by Scott & Pease (1956), Gonzales & Karnovsky (1961), and Hancox & Boothroyd (1961) Woods & Nichols (1965) found some fractions of rat bones to be rich in collagenase activity Goldhaber (1961) demonstrated that resorption of bone was followed by degradation of bone collagen. One or several enzymes are released during the resorption of bone (Stern, 1965) and this or these enzymes have collagenolytic activity The studies of Kaufman et al (1965) confirmed that active resorption of bone in tissue cultures is followed by a release of a collagenolytic factor capable of breaking down collagen fibrils at a neutral pH and 37 C. This process has been found to be increased by parathyroid extract (Walker et al., 1964) and heparin.

The disagreement between the osteoclastic resorption theory and the enzyme theory which we are advancing here—and which can explain our light microscopic as well as electron microscopic findings—is perhaps more apparent than real. It is known that when the osteoclasts act locally through their ruffled borders and absorb calcium hydroxyapatite crystals by pinocytosis, they may also exert an important acid phosphatase activity (Schajowicz & Cabrini, 1958 Burstone 1959 1962 Handelman et al 1964) an activity which perhaps plays a role at a certain distance from the cell, although this has never been demonstrated.

The enzymatic lysosomal theory we are advancing is based upon morphological as well as experimental findings. Morphologically as previously mentioned, the regular centrifugal extension of large destruction foci (in the way of a front") as well as the findings of small foci splashing" around can only be explained by an enzymatic process. The presence of cells showing in their cytoplasm a varying number of lysosomal, dense bodies, and which we have called histiocytes (although names are not the cardinal point) guarantee the enzymatic material needed for the bone resorption. The activity of the numerous hydrolytic enzymes found in the lysosomes explains—by reason of the protease, hyaluronidase, cathepsin, and acid phosphatase content—why destruction of the tissue may take place, although it is, as in this case, a dense bony tissue with abundant collagen. In addition, we have demonstrated the extension of cell destruction caused by changes in the permea-

bility of the lysosomal membrane Extrusion of material, or right out cell destruction will in both events release hydrolytic material When the activity of the acid phosphatases is used as a cytochemical index, this hydrolytic material has proved to be active both within the cell, immediately around the cell lacuna, in the extension of the focus, and in microfoci In this way the enzymatic theory can explain the destruction, its special course and the extension process

Thus, we have brought otosclerosis research on a cellular basis Thereby we can correlate the pathological condition to a number of important *in vitro* studies of embryonic bone and cartilage cells For instance, it has been reported by Sledge (1965) and by Vaes (1964 1965) that under different conditions, such as an increase in the oxygen tension to the cells or administration of parathyroid hormone, the hydrolytic lysosomal activity will increase and result in resorption of the culture On the basis of our morphological and experimental studies we believe that this is what happens in the otosclerotic focus. During the lytic phase irrespective of its cause—be it e g. pregnancy inflammation, traumas, irradiation, etc —the *lysosomal hydrolyses make up a common* denominator in the otosclerotic resorption.

10 Summary in French

Les auteurs soulignent dans leur introduction l'énorme fossé qui sépare actuellement les remarquables résultats du traitement chirurgical de l'ignorance qui caractérise encore la compréhension biologique de l'otospongiose Ils insistent sur la nécessité d'une étroite collaboration entre chirurgiens et chercheurs qui seule permet de placer l'étude de la maladie otospongieuse sur un plan véritablement biologique c'est à-dire cellulaire et enzymatique

Dans un deuxième chapitre sont résumés les études biochimiques et histochimiques concernant la maladie Les auteurs indiquant que les études biochimiques portant sur l'analyse des fluides organiques, les dosagen hormonaux etc n'ont jusqu'ici fourni que des résultats assez contradictoires par contre les résultats des rares études histochimiques sont plus cohérents et concordent pour assigner un rôle de premier ordre aux mucopolysaccharides et au collagène de même qu'ils s'accordent pour reconnaître au niveau de foyer otospongieux une augmentation des enzymes respiratoires et des activités phosphatisiques alcalines et acides.

Le troisième chapitre est consacré à un résumé de nos connaissances actuelles concernant le rôle des phosphatases dans le processus complexe de l'ossification et de la destruction du tissu osseux, processus que l'on retrouve tout au long de la longue évolution de l'otospongiose

Puis les auteurs fournissent des précisions sur leur matériel et les techniques utilisées. Ils ont étudié 14 « lamelles ostéoïdes » et 50 fragments osseux d'étriers, tous fixés immédiatement après leur prélèvement chirurgical (fixation classique par la glutaraldehyde à 2% en tampon cacodylate 0 1 M) La décalcification lorsqu'elle s'avérait nécessaire fut effectuée en 12 h à froid dans une solution d'EDTA à 4,5 % de façon à ce que l'osmolarité de toutes les solutions soient situées aux environs de 440 millosmoles

La recherche enzymatique (phosphatase acide) est effectuée suivant la technique de Gomori légèrement modifiée puis une post fixation osmique est pratiquée Les coupes obtenues après section furent colorées uniquement à l'acétate d'uranyl Les résultats observés peuvent être résumés ainsi

Durant la phase destructive ou de lyse la microscopie électronique révèle des foyers extensifs, sans doute provoqués par la coalescence de plusieurs zones de lyse Ces foyers sont

caractérisés par leurs bords nettement tranchés, par l'existence très fréquente en leur voisinage immédiat de microfoyers dont la taille ne dépasse pas quelques microns et qui semblent bien précéder le front d'extension de la maladie. En outre, lorsque la destruction tissulaire est encore en cours, un aspect très caractéristique est réalisé par l'effilement et la perte de striation caractéristique des très nombreuses fibres collagènes de l'os, les auteurs le décrivent sous le nom d'aspect en « pipette Pasteur

L'aspect des cellules est discuté. En particulier les auteurs soulignent le fait que certains ostéocytes sans être touchés par le processus otospongieux offrent des aspects dégénératifs qui font partie des aspects métaboliques normaux de la capsule otique

En outre au sein des foyers de lyse on trouve des cellules auxquelles on attribue une valeur histiocytaire. Ces cellules semblent jouer dans le processus de lyse un rôle plus important que les ostéoclastes qui sont classiquement tenus pour responsables des processus de destruction. Une caractéristique capitale de ces cellules est leur richesse en lysosomes. La valeur physiologique de ces vésicules limitées par une seule membrane dont le contenu est dense aux électrons est maintenant connue On leur attribue essentiellement un rôle de stockage des principales enzymes hydrolytiques de même qu'un rôle de digestion des corps étrangers et molécules phagocytées. Il résultate de ces données que la rupture de leur membrane peut provoquer la destruction des tissus péricellulaire ainsi qu'éventuellement la mort de la cellule. Les mêmes constatations cytologiques furent effectuées au niveau des lamelles ostéoïdes de Causse qui apparaissent comme des foyers d'os otospongieux en situation intramuqueuse et juxta focales.

La théorie enzymatique proposée par les auteurs et qui fait des lysosomes l'élément principal de la phase destructive de l'otospongiose a été confirmée par la recherche de l'activité phosphatasique acide au niveau du foyer otospongieux, on reconnaît en effet très généralement à cette enzyme une valeur d'index des différentes activités hydrolytiques normalement contenues dans les lysosomes

Les auteurs ont mis en évidence au microscope électronique une activité phosphatasique acide en des localisations très précises de l'os otospongieux.

1 — au niveau d'ostéocytes ou d'histiocytes en voie de destruction (en particulier au niveau des zone de lyse intracytoplasmique consécutives à la rupture des vésicules lysosomales)

2. — au niveau des logettes cellulaires et des canalicules qui en partent.

3 — au niveau de front d'extension des foyers otospongieux actifs (l'étroite bande d'activité enzymatique mordant toujours sur le tissu normal)

4 — au sein des microfoyers qui précèdent le front d'extension

En conclusion

Les auteurs proposent pour expliquer la phase de lyse du foyer otospongieux, la moins bien connue sans aucun doute une théorie enzymatique qui fait appel à l'activité hydrolytique du contenu des lysosomes. Cette théorie explique parfaitement les constatations morphologiques tirées de l'étude électronmicroscopique et qui caractérisent cette phase de lyse.

11 Summary in German

Die Verfasser unterstreichen in der Einleitung ihrer Veröffentlichung die *enorme Kluft*, die zur Zeit zwischen den bemerkenswerten Ergebnissen der operativen Behandlung der Otospongiose und der Unkenntnis, die noch das biologische Verständnis dieser *Krankheit* charakterisiert, besteht. Sie legen Nachdruck auf die Notwendigkeit einer engen Zusammenarbeit zwischen Chirurgen und Forschern allein eine solche erlaubt es, die Untersuchungen dieses

Leidens auf ein echt biologisches, d h ein zell und enzymatisches Gebiet zu leiten

Im zweiten Abschnitt werden die bio- und histochemischen Studien dieser Krankheit zu sammengefasst Die Verfasser weisen darauf hin, dass die biochemischen Untersuchungen, die die Analyse der organischen Flüssigkeiten die Hormonbestimmungen usw betreffen, bis jetzt nur ziemlich widersprechende Ergebnisse lieferten Im Gegensatz dazu zeigen die raren histochemischen Resultate besseren Zusammen hang und stimmen darin überein den Muko-polysacchariden und Kollagenen eine Rolle er sten Ranges einzuräumen, ebenso wie sie sich einig sind um im Bezirke des otospongiosen Krankheitsherdes eine Zunahme der Atmungs-fermente und der alkalischen und sauren Phos phatase-Aktivitäten zu erkennen

Das dritte Kapitel ist eine Zusammenfassung der heutigen Kenntnisse in der Bedeutung der Phosphatasen für den komplexen Verlauf der Ossifikation und der Destruktion der Knochen gewebe, diesen Prozess findet man im Laufe der ganzen, langen Entwicklung der Otospongi ose wieder

Anschliessend legen die Verfasser Angaben über ihr Material und die angewandten Tech niken vor Sie haben 14 „Osteoidplättchen und 50 vom Steigbügel herrührende Knochenstück chen untersucht alle waren sofort nach der operativen Entfernung fixiert worden (übliche Fixierung mit 2%ig. Glutaraldehyd — als Puf fer Kakodylate 0 1 M) Wenn eine Entkalkung nötig war wurde sie in kaltem Zustand in einer 4,5% igen EDTA Lösung vorgenommen (12 Stunden) sodass das isotonische Gleichgewicht aller Lösungen ungefähr bei 440 mOsmol lag.

Die enzymatische Untersuchung (saure Phos-phatase) wurde mit Hilfe der Gomori Methode durchgeführt, die eine leichte Abänderung er fahren hatte anschliessend wurde eine Nach fixierung mit Osmiumsäure vorgenommen. Die gewonnenen Schnitte wurden nur mit Uranyl acetat gefärbt

Die gesammelten Erfahrungen kann man folgenderweise zusammenfassen. Während der destruktiven Phase oder der Lyse bringt die Elektronenmikroskopie extensive Herde an den Tag, die ohne Zweifel durch Zusammenballung mehrerer Lysis Bereiche hervorgerufen wurden Diese Herde zeichnen sich durch ihre scharf abstechenden Ränder aus, sowie durch die in ihrer unmittelbaren Nähe sehr häufige Anwesen-heit von Mikroherden deren Grösse einige Mikronen nicht überschreitet, und die — wie es scheint — der „Ausbreitungsfront dieser Krankheit vorangehen

Weiter entsteht noch während der Gewebs zerstörung ein sehr charakteristisches Bild durch Zerfaserung und Verlust der charakteristischen Streifung der sehr zahlreichen kollagenen Fa-sern im Knochen. Die Verfasser beschreiben dieses Bild unter der Bezeichnung „Pasteur Pipette Das Aussehen der Zellen wird be sprochen Die Verfasser heben besonders die Tatsache hervor nach der verschiedenen Osteo-cyten degenerative Erscheinungen aufweisen ohne von otospongiosen Prozessen befallen zu sein diese Erscheinungen gehören zum norma-len metabolischen Bild der Labyrinthkapsel.

Ausserdem findet man inmitten der Lyse-Herde Zellen, denen man einen histiozytären Wert zuschreibt Diese Zellen scheinen im Pro-cessus der Lyse eine bedeutendere Rolle zu spielen als die Osteoklasten die klassich für den Zerstörungsprozess verantwortlich gemacht werden. Ein wesentliches Kennzeichen dieser Zellen ist ihr Reichtum an Lysosomen Der physiologische Wert dieser von einer einzigen Membran begrenzten Bläschen, deren Inhalt elektronenundurchlässig ist, ist jetzt bekannt Man schreibt ihnen vor allem die Rolle der Speicherung der hauptsächlichsten hydrolyti schen Enzyme zu, sowie die der Verdauung der fremden Körperchen und der Phagozytenmole küle Aus diesen Angaben geht hervor dass das Zerreissen ihrer Membran die Zerstörung der perizellulären Gewebe hervorrufen kann, sowie eventuell auch das Absterben der Zelle Die selben zytologischen Verhältnisse wurden im Bereich der Causse Osteoidplättchen festge-stellt, die wie Herde otospongioser Knochen in intramuköser Lage in Erscheinung treten

Die enzymatische Theorie die von den Ver

'assern vorgeschlagen wird und die die Lysosome als hauptsächlichstes Element der destruktiven Phase der Otospongiose betrachtet, wurde durch die Untersuchung der sauren Phosphatase-Aktivität im Bereich des otospongiosen Herdes bestätigt. Man schreibt tatsächlich im allgemeinen diesem Ferment einen „Indexwert" der verschiedenen hydrolytischen Aktivitäten zu, die normalerweise in den Lysosomen vorhanden sind.

Die Verfasser haben mit Hilfe der Elektronenmikroskopie eine saure Phosphatase Aktivität nachgewiesen und die genaue Lokalisierung dieser im otospongiosen Knochen angegeben.

1 im Bereich der sich in Zerstörung befindenden Osteozyten oder Histiozyten (besonders im Bereich der intrazytoplasmatischen Lyse-Bezirke, als Folge einer Ruptur der Lysosomenbläschen),

2. im Bereich der Zellenlagunen und der von diesen ausgehenden Kanälchen,

3 im Bezirk der „Ausbreitungsfront" der aktiven otospongiosen Herde (der schmale Streifen mit Enzymaktivität greift immer auf das normale Gewebe über)

4 inmitten der Mikroherde, die der „Ausdehnungsfront" vorausgehen.

Schlussfolgerung

Um die am wenigsten bekannte Phase der Lysis im Otospongiose Herd klarzustellen, legen die Verfasser eine enzymatische Theorie vor die sich auf die hydrolytische Aktivität des Lysosomeninhalts beruft. Diese Theorie erklärt die morphologischen Befunde vollkommen, die durch Untersuchungen am Elektronenmikroskop gewonnen wurden und die für diese Lyse characteristisch sind.

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SUPPLEMENT 274

A Provocative Test for Grading Susceptibility to Motion Sickness Yielding a Single Numerical Score

BY

E. F MILLER II, PH.D and A. GRAYBIEL, M D

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Introduction

Individual differences in motion sickness susceptibility have been scored by a pass-fail dichotomy designed primarily for screening highly susceptible individuals and have been ranked according to the level of response to some type of sustained stimulus. However no known method has yielded a single numerical score that would permit comparison of individuals on a continuous scale of susceptibility values. Such a procedure has two major requirements (1) standardization and quantitative definition of symptoms that are reliably diagnostic of a specific level of motion sickness, (2) choice of a stimulus that is effective for the majority of normal subjects and which, for practical purposes, can be generated by simple or conventional apparatus and be readily measured.

The endpoint favored by most investigators of motion sickness susceptibility is the demonstration of frank sickness, viz. severe nausea or vomiting. Such a criterion has certain disadvantages, however in that physiological as well as psychological complications can affect serial measurements (e.g., drug or habituation studies). A more acceptable and definitive approach considers diagnostic criteria based upon symptomatology manifested prior to this severe expression of motion sickness. A guideline for such an approach has recently been proposed (Graybiel et al., 1968) which, in quantitative terms, categorizes specific symptoms and identifies five levels of severity of motion sickness Each of the four endpoints short of frank sickness allows the experimenter to stress his subjects to a nearly equivalent extent without the manifestation of severe symptoms This choice in the test design of an equivalent response criterion, instead of a common schedule of physical forces for all subjects, was made to gain greater differentiation of individual differences, as well as to spare highly susceptible individuals from undue stressor effects and to eliminate the possibility of regarding more resistant ones as immune to motion sickness.

Of the various available means of experimentally provoking symptoms of motion sickness, standardized head (body) movements during constant speed rotation in a rotational chair represent a convenient and highly effective method (Dowd, 1965 Harris et al., 1963 Kraus 1960 Lansberg, 1954 Khilov 1969 Spiegel et al., 1944). The present study evaluated a variation of this general method, but one which was designed specifically to measure individual susceptibility along a common scale of stress with an equal endpoint. The dependent variable was a quantitatively defined malaise level and the independent variable the physical dimension of a standardized Coriolis stimulus

Procedure

Subject

The normal group consisted of 250 men, of whom 193 were aviation students or flight crew personnel the remaining 57 were comprised of 11 nonaviator officers, 41 enlisted men, and 5 civilians with flying assignments. Their ages ranged from 16 to 43 years all but 18 were between 19 and 26 years of age.

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Table II *Rotary chair test velocities most often associated with average experience and symptom levels coded from Motion Experience Questionnaires*

	Symptoms S										
Experience X	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
0.5	10.0*	10.0	10.0	10.0	10.0	10.0	10.0	7.5	5.0	5.0	5.0
1.0	12.5	12.5	10.0	10.0	10.0	10.0	10.0	7.5	5.0	5.0	5.0
1.5	12.5	12.5	12.5	10.0	10.0	10.0	10.0	7.5	7.5	5.0	5.0
2.0	12.5	12.5	12.5	12.5	12.5	10.0	10.0	10.0	7.5	5.0	5.0
2.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	10.0	7.5	5.0	5.0
3.0	15.0	15.0	12.5	12.5	12.5	12.5	12.5	12.5	10.0	7.5	7.5
3.5	20.0	15.0	15.0	15.0	12.5	12.5	12.5	12.5	10.0	7.5	7.5
4.0	25.0	20.0	15.0	15.0	15.0	15.0	12.5	12.5	10.0	7.5	7.5
4.5	30.0	25.0	20.0	20.0	20.0	15.0	15.0	12.5	10.0	7.5	7.5
5.0	30.0	30.0	25.0	25.0	25.0	20.0	15.0	12.5	10.0	7.5	7.5

* Rotary chair velocity (rpm).

he was secured by a lap belt in the Stille rotary chair and a blindfold was put over his eyes to eliminate any visual influences.

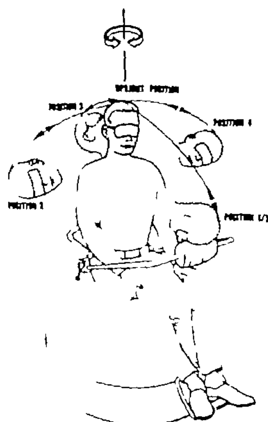


Fig. 1. Diagram of standardized procedure for making each sequence of head movements to and from tilt position 1 through 5 during chair rotation.

While stationary the subject was required to demonstrate the standardized head movement sequence which would provide the Coriolis accelerations during chair rotation: front, upright, pause; right, upright, pause; back, upright, pause; left, upright, pause; front, upright, rest (Fig. 1). Each 90° tilt movement or the return to upright was executed smoothly over a 1-sec period. The pauses between movements were of the same (1 sec) duration, with the final pause (rest) lasting for 20 sec. A taped recording was used to standardize the temporal sequence of head movements.

With the subject in an upright position, the chair was accelerated 5°/sec² either in the clockwise or counterclockwise direction, selected at random, until one of several constant velocities was reached (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0 rpm). Sixty seconds later the test was begun with the first head movement sequence. The sequences were continued until the cumulative point score of the symptoms totaled at least 8, the lowest number of points in the M III criterion (Graybiel et al., 1968). Immediately upon manifesting M III the subject terminated his head movements and returned to his upright position, the chair was decelerated at 5°/sec² to a stop. Specific motion sickness signs and symptoms were scored on a tally sheet (Appendix C) as they appeared. With this aid, even an observer with only a minimal amount of training could record the

Table I *Diagnostic categorization of different levels of severity of acute motion sickness*

Category	Pathognomonic 16 points	Major 8 points	Minor 4 points	Minimal points	AQS[a] 1 point
Nausea syndrome	Vomiting or retching	Nausea II III[b]	Nausea I	Epigastric discomfort	Epigastric awareness
Skin		Pallor III	Pallor II	Pallor I	Flushing/Subjective warmth ≥ II
Cold sweating		III	II	I	
Increased salivation		III	II	I	
Drowsiness		III	II	I	
Pain					Headache ≥ II
Central nervous system					Dizziness Eyes closed ≥ II Eyes open III

Levels of severity identified by total points scored

Frank sickness (FS)	Severe malaise (M III)	Moderate malaise A (M IIA)	Moderate malaise B (M IIB)	Slight malaise (M I)
≥ 16 points	8–15 points	5–7 points	3–4 points	1 2 points

[a] AQS = additional qualifying symptoms.
[b] III = severe or marked, II = moderate, I = slight.

tion required by the Navy Department, all subjects were given specific tests of otolith (ocular counterrolling) (Miller II 1961 1966) and semicircular canal function (caloric threshold) (McLeod & Meek, 1962) and oculogyral illusion threshold (Graybiel & Hupp 1946) Each of the normal subjects manifested vestibular responses which were well within normal limits

Three completely deaf persons with total or severe bilateral loss of semicircular canal and otolith function (Miller II & Graybiel 1963) acted as control subjects

Method

The procedure for measuring motion sickness susceptibility to Coriolis forces included a pretest evaluation of the subject for individual selection of stimulus level (chair velocity) and general fitness a simple method of scoring diagnostic criteria of motion sickness (Table I) and the grading of an individual in terms of a quantitative measure (index) of Coriolis sickness susceptibility derived from the cumulative head movements executed at a given chair velocity

The predetermined endpoint for each subject was severe malaise (M III) The desired level of motion stress imposed upon each subject was such that this level of malaise was approached rather gradually so that the observer could readily identify and register symptoms in sequence as they were manifested, but more importantly so that the subject was not overstimulated, particularly to the point of extreme nausea or vomiting (frank sickness) For these reasons, the Motion Experience Questionnaire (MEQ) (Appendix A) based on the Pensacola Motion Sickness Questionnaire (Hardacre & Kennedy 1963) was used in conjunction with Table II as the basis for selecting the rotational rate for testing each subject.

Table II lists the best estimate of the chair's rotational test rate (rpm) which we were able to gain empirically from the average level of experience (X) and intensity of symptoms (S) reported in the MEQ Usefulness of Table II is demonstrated by the fact that, by this table an rpm could be predicted which yielded M III in approximately 80% of the 250 subjects at between 40 and 166 head movements.

The subject's fitness for testing was established from his completed Preexperimentation Questionnaire (Appendix B) After both questionnaires were evaluated the subject was briefed on the symptoms he could expect. Then

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4.0	25.0	20.0	15.0	15.0	15.0	15.0	12.5	12.5	10.0	7.5	7.5
4.5	30.0	25.0	20.0	20.0	20.0	15.0	15.0	12.5	10.0	7.5	7.5
5.0	30.0	30.0	25.0	25.0	25.0	20.0	15.0	1_5	10.0	7.5	7.5

[a] Rotary chair velocity (rpm)

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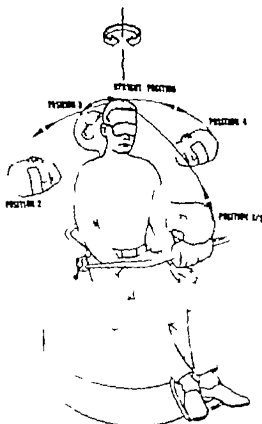


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symptomatology efficiently and stop the test precisely when the endpoint was reached.

The Coriolis sickness susceptibility index

The stress effect of a standard head tilt as a function of chair velocity was measured in another study (Miller II & Graybiel 1970) by determining among several subjects the number of head tilts required to elicit a common malaise level at each of several different chair velocities Individually the regularity of this function was limited to rotational rates above a critical amount, that which apparently stressed the subject beyond his functional vestibular reserve (FVR) (Graybiel, 1969) When the rpm was reduced below this point there was characteristically a sudden marked increase in the subject s capacity for making head movements without evoking symptoms.

When head movements at a given chair velocity introduce vestibular stress in excess of the FVR, the average relative stimulus effect of a single head movement[1] can be expressed by the factor E which is linearly related (log/log function) to chair velocity (Fig 2) (Miller II & Graybiel 1970) Each individual's score, referred to as his *Coriolis Sickness Susceptibility* Index or CSSI therefore can be calculated simply by multiplying the appropriate E factor for the rpm used in his test by the number of head movements (N) required to elicit M III

$$\mathrm{CSSI} = E \times N$$

The resultant value expresses quantitatively motion sickness susceptibility to Coriolis acceleration within a single convenient scale of numbers (0 to 100)

It was found in the program of this test s development that when M III occurred within the range of 40 to 166 head movements the signs and symptoms developed regularly and gradually without the hazard of provoking frank sickness If M III was not reached within this range the test was considered invalid and the subject was retested after at least 48 hours had elapsed Any subject tested at 30 rpm who failed to reach the endpoint was not retested since his performance was considered to indicate essential immunity to Coriolis sickness In the retest the chair was rotated in the opposite direction from that of the first test and at a different rpm that was based on the subject s response in the initial test The duration of each test was usually less than 15 min

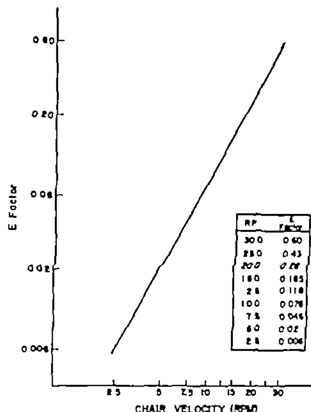


Fig 2 Relationship between average relative stress effect (E factor) of a single head movement and rotational velocity

Test retest evaluation

Thirty unselected subjects whose susceptibility level (CSSI score) had been properly measured were retested at the same rotational rate to determine test retest reliability The standard Spearman (rank) method revealed the degree of relationship between the rankings of the individual CSSI scores calculated for the two test sessions

[1] Head movement in the four directions as required in this test is not equally stressful (Guedry & Montague, 1961 Ichiro et al., 1963) and the effect of direction of movement varies among individuals and occasionally even in the same individual

Results

NORMAL SUBJECTS

Symptomatology

The frequency with which each of the diagnostic categories of symptoms appeared among the normal group at the level of M III is presented in Fig. 3

The incidence of each category of symptoms, which in most cases were classified as mild (I), moderate (II) or severe (III) was as follows epigastric awareness, epigastric discomfort, or nausea I, 90.4% pallor (I II) 84.4% cold sweating (I, II, III), 66.8% flushing/subjective warmth (≥II) 72.4% Increased salivation (I, II) 37.2% dizziness (≥II) 25.6% drowsiness (I, II) 21.6% headache (≥II) 1.2%

Use of the M III criterion as an endpoint in this test prevented the evocation of severe levels of increased salivation, pallor or drowsiness, only four subjects manifested cold sweating III Nausea did not exceed the mild level, and in fact 9.6% of the subjects remained completely free from any epigastric involvement whatsoever

Table III. *Coriolis Sickness Susceptibility Index (CSSI) values represented by various percentile scores*

CSSI	Per cent	CSSI	Per cent
1.1	1	11.3	60
3.2	5	12.9	70
4.7	10	16.0	80
6.2	20	26.0	90
7.2	30	58.0	95
8.6	40	91.5	99
10.0	50		

Coriolis sickness susceptibility index

The distribution of Coriolis Sickness Susceptibility Index values for all subjects is plotted in Fig. 4 The values ranged from 0.4 to 100.0 but the distribution is markedly right skewed (mean = 15.3 median = 10.0 mode 7–8) 90% of this population fell within 0.4 and 26.0 points Table III lists CSSI values in terms of percentile scores.

The schedule of chair velocities was found to be adequate to test a wide spectrum of suscepti-

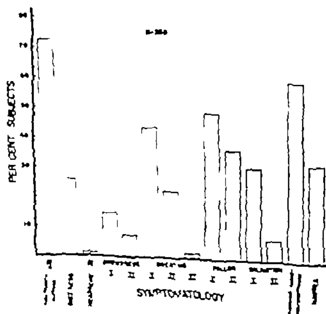


Fig. 3 Frequency of occurrence among 250 normal subjects of specific diagnostic symptoms associated with Malaise III.

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When head movements at a given chair velocity introduce vestibular stress in excess of the FVR, the average relative stimulus effect of a single head movement[1] can be expressed by the factor E which is linearly related (log/log function) to chair velocity (Fig 2) (Miller II & Graybiel 1970) Each individual's score referred to as his Coriolis Sickness Susceptibility Index or CSSI therefore, can be calculated simply by multiplying the appropriate E factor for the rpm used in his test by the number of head movements (N) required to elicit M III

$$\text{CSSI} = E \times N$$

The resultant value expresses quantitatively motion sickness susceptibility to Coriolis acceleration within a single convenient scale of numbers (0 to 100)

It was found in the program of this test's development that when M III occurred within the range of 40 to 166 head movements the signs and symptoms developed regularly and gradually without the hazard of provoking frank

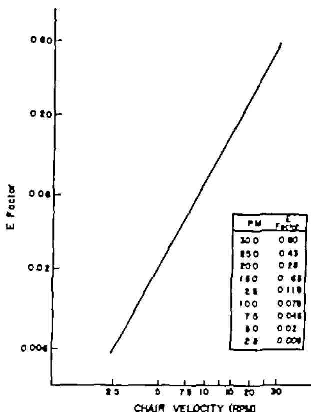


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sickness If M III was not reached within this range the test was considered invalid and the subject was retested after at least 48 hours had elapsed Any subject tested at 30 rpm who failed to reach the endpoint was not retested since his performance was considered to indicate essential immunity to Coriolis sickness In the retest the chair was rotated in the opposite direction from that of the first test and at a different rpm that was based on the subject's response in the initial test The duration of each test was usually less than 15 min

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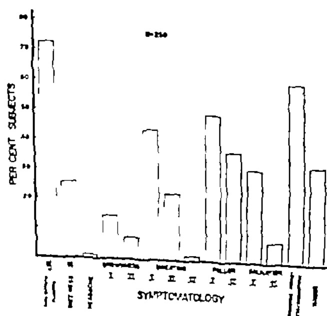


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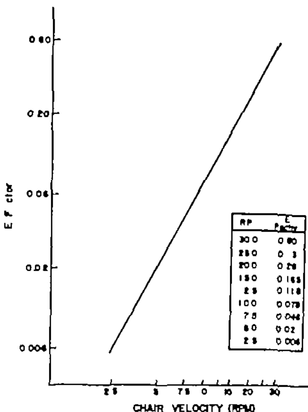


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sickness If M III was not reached within this range, the test was considered invalid and the subject was retested after at least 48 hours had elapsed Any subject tested at 30 rpm who failed to reach the endpoint was not retested since his performance was considered to indicate essential immunity to Coriolis sickness In the retest the chair was rotated in the opposite direction from that of the first test and at a different rpm that was based on the subject s response in the initial test The duration of each test was usually less than 15 min

Test-retest evaluation

Thirty unselected subjects whose susceptibility level (CSSI score) had been properly measured were retested at the same rotational rate to determine test retest reliability The standard Spearman (rank) method revealed the degree of relationship between the rankings of the individual CSSI scores calculated for the two test sessions

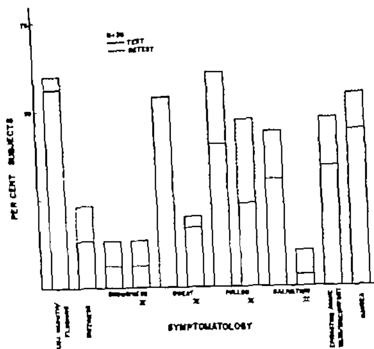


Fig. 6. Similarity of symptomatology patterning between the test and retest of Coriolis susceptibility of 30 normal subjects.

Discussion

The index of Coriolis sickness susceptibility is valid only within certain limits of the number of head movements which, in turn, are dependent upon the chair's rotational rate. Six subjects (A, B, C, D, E, F, Fig. 7) were selected from the 250 normal ones to serve as examples of the difference in the rate of the buildup of symptoms and to illustrate the need for careful selection of chair velocity. An ideal type of response in terms of rate of symptom buildup falls near to or within the limits represented by subjects D and E. As a rule, it was extremely difficult, if not impossible, at times to prevent a skyrocketing of symptoms up to the level of frank sickness (FS), as illustrated by subjects A and B who manifested the typical response obtained when the rpm is too high for the individual. If, on the other hand, the rpm selected for him is too low, he can continue to make head movements without provoking any symptoms (subject F). There were other subjects who at first displayed mild symptoms, but these decreased and disappeared as the test progressed; however, when each of these subjects was retested at an rpm which yielded a stressor condition above that for which he could compensate, a pattern of response similar to that of subjects D and E was seen. The rest period between head movement sequences was found to be short enough so that any appreciable recovery from previous vestibular Coriolis stimulation did not occur, yet it allowed for the characteristic lag in the appearance of motion sickness symptoms after each exposure to a head movement sequence.

The distribution of Coriolis sickness susceptibility index (CSSI) values among the population of 250 unselected normal subjects of this study revealed that most normal individuals are moderately or highly susceptible to Coriolis stress; therefore, the suggested binomial distribution function (Brand & Perry 1966) is inappropriate. The fact that our population of subjects was formed predominantly of flight

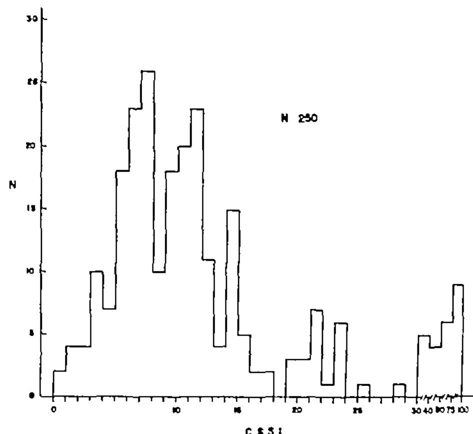


Fig 4 Distribution of Coriolis Sickness Susceptibility Index (CSSI) among 250 normal subjects.

bility to Coriolis motion sickness in this population, although the maximum rotational rate did not provoke symptoms in three of the 250 subjects

Test Retest Reliability The Coriolis Sickness Susceptibility Index of 30 subjects as determined with the M III criterion in test and retest sessions correlated highly ($\rho = 0.89$) A scattergram plot (Fig. 5) reveals that none of the subjects changed substantially in his level of susceptibility from one session to the next.

The patterning of symptoms for the group was remarkably similar (Fig. 6) in these two test sessions, and, individually almost identical symptoms in terms of number type and intensity were provoked in the majority of cases

LABYRINTHINE DEFECTIVE (L D) SUBJECTS

None of the L D subjects experienced even the slightest symptom or unpleasant feeling during or following the execution of up to 300 head movements at the highest rotational velocity of the chair (30 rpm)

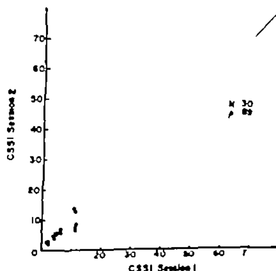


Fig 5 Scattergram of test vs. retest Coriolis Sickness Susceptibility Indices (CSSI) of 30 normal subjects.

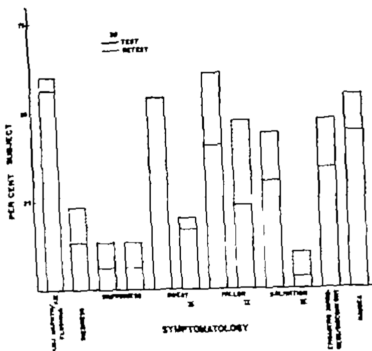


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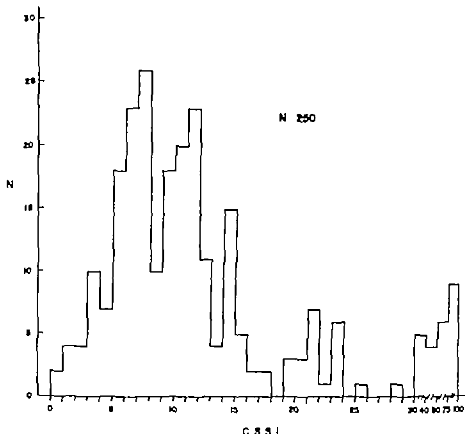


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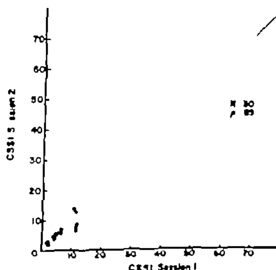


Fig 5 Scattergram of test vs. retest Coriolis Sickness Susceptibility Indices (CSSI) of 30 normal subjects.

ferent directions for a limited number of times, covering the eyes, and if the test was repeated, reversing the direction of rotation (CW CCW) were procedures introduced to increase the complexity of the stimulus, and to decrease experiential factors, thereby reducing the subject's ability to habituate to the test conditions. Furthermore, a chair velocity was carefully selected which would stress the individual at a level greater than his capability for making compensatory adjustments, i.e., above his functional vestibular reserve. These procedures probably contributed to the high test-retest reliability in this and in a preceding study (Miller II et al. 1969).

The stability of the results which are expressed quantitatively within a single scale of values, renders this test highly useful in specifying individual susceptibility as well as in determining the influence of a variety of factors (e.g., drugs, training) upon this basic measurement. Simplicity of the test, the short time period required, and use of apparatus commonly found in a vestibular laboratory are practical advantages.

Zusammenfassung

Eine Standardmethode für Quantifizierung der Empfänglichkeit für Coriolis (See) Krankheit wurde mit 250 normalen und drei Labyrinthdefekten Versuchspersonen ausgewertet. Die Versuchsperson musste eine bestimmte Kopfbewegung (±90° in der Front und Seitenebene) ausführen, während sie in einem Stuhl sass, der mit jeweils einer von verschiedenen konstanten Geschwindigkeiten rotierte. Die geeignete Versuchsgeschwindigkeit wurde vorhergesagt in der Mehrzahl der Fälle mit Hilfe des Motion Experience Fragebogens. Drei normale und alle Labyrinth-defekten Personen erwiesen sich als unempfänglich bei diesen Versuchsbedingungen. Der Seekrankheitsempfänglichkeit Index CSSI wurde für jede Versuchsperson bestimmt durch Multiplizierung des gefundenen E Faktors, d. h. des mittleren Stressfaktors für jede Kopfbewegung bei der Umdrehungszahl des Versuches, mit der Zahl der erforderlichen Kopfbewegungen, um starke Malaise zu erzeugen (M III). Die gefundenen CSSI *Werte für* die 250 Versuchspersonen lagen im Bereich von 0.4 bis 100, doch war die Verteilung deutlich nach rechts verschoben. Das Verfahren ergab eine hohe Reproduzierbarkeit ($\rho = 0{,}89$) der CSSI Werte und im Symptomenablauf. Wenn der Malaise III Grad erreicht wurde, zeigte sich in den meisten Fällen das Übelkeitssyndrom, doch blieb ein signifikanter Prozentsatz (9,6%) der Versuchspersonen frei von epigastrischer Störung oder Übelkeit.

References

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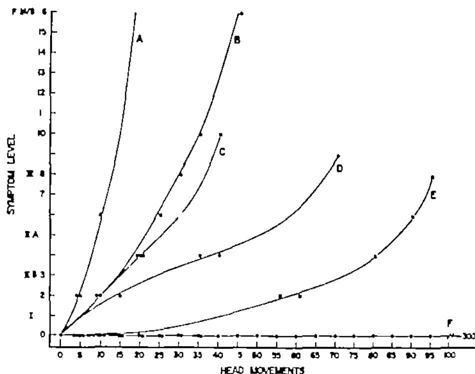


Fig 7 Variations in the rate of symptom buildup in response to head movement as illustrated by six selected subjects (*A* through *F*)

personnel would seem to indicate that substantial adverse response to Coriolis acceleration would be the rule rather than the exception in the general population

The essentiality of the vestibular organs in the genesis of motion sickness was again demonstrated by the fact that the subjects lacking the function of these organs remained symptomless when exposed to the severest Coriolis acceleration provided by this test. On the other hand, the fact that three of the subjects with confirmed normal vestibular function were similarly resistant to the same stressful conditions reveals that the corollary is not always true and demonstrates the marked individual differences in susceptibility which occur among normal subjects

With the aid of one minor qualification, the diagnostic criteria for categorizing different levels of severity of acute motion sickness as reported previously (Graybiel et al., 1968) served without exception in quantitatively grading the susceptibility of all subjects The need to alter the original schema was revealed when a large percentage (72 4%) of the 250 subject group (Fig 3) reported an acute increase in apparent body warmth of ≥ II intensity which was occasionally but not usually accompanied by flushing, the objective counterpart of elevated skin temperature For this reason a moderate or greater increase in the subject's feeling of warmth ("subjective warmth") was regarded in this test as equivalent to flushing and, singly or in combination with flushing was identified as an Additional Qualifying Symptom (AQS) with a value of a single point.

Either nausea I epigastric discomfort, or epigastric awareness was the predominant feature of severe malaise (III) However a proportion of the test population (9 6%) failed to manifest even the mildest form of gastrointestinal disturbance This finding is not in agreement with the classical viewpoint which for the most part equates motion sickness with a gastrointestinal reaction marked by nausea or vomiting. If M III as diagnosed by a nonnausea symptom complex is equivalent in terms of the subject's well being his psychomotor efficiency or some other indicator to that involving the nausea syndrome then the restricted "nausea syndrome" criterion of motion sickness must be reevaluated

Attention was given in the design of this test to factors which would reduce or if possible eliminate habituation Moving the head in dif

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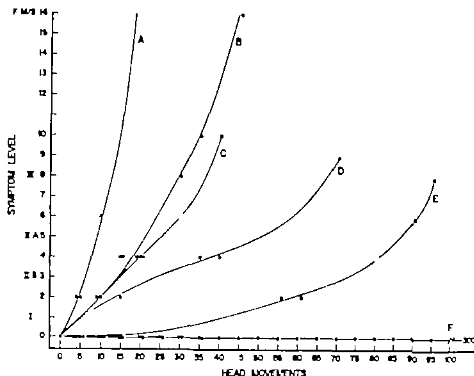


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Appendix A

MOTION EXPERIENCE QUESTIONNAIRE INSTRUCTION FOR THE EXAMINER

In attempting to evaluate motion sickness susceptibility based upon this (historical) account by the subject, two primary factors are used. (1) type and number of exposures to motion and (2) the effects in terms of the average intensity of symptoms which were recalled in these experiences. These factors, the subject's experience (X) and intensity of symptoms (S) for each of the motion environment categories, are identified in the Questionnaire by X and S and coded on a five-point scale. If symptoms are indicated for the "Swings and other Gymnastic Equipment" category (page 19) its X and S values are used in the calculations, otherwise this category is omitted entirely. The fact that this category is infrequently used increases its significance when filled in, and the X and S values are arbitrarily weighted by a factor of 2×. The average of the 9 (or 10) X and 10 (or 11) S values are used in conjunction with a table that lists the appropriate empirically derived estimate of the chair velocity to be used in testing each subject.

MOTION EXPERIENCE QUESTIONNAIRE

PART I

Name ______________ Date, Mo ____ Da ____ Y ____ Age Yrs __ M __

Serial No. ________ Rank or Rate ____________ Designator ____________

Circle: Male or Female Date of birth ________ Weight ________ Height ________ Handedness R L

Referral source ______________________________

Referral problem and/or diagnosis ______________________________

Circle one or more of the following:

Navy Marine Air Force Coast Guard Civilian

Astronaut Aviator Navigator Flight Surgeon Aircrewman

Line Officer Staff Corps Officer Student Aviator AOC Enlisted Other (Specify) ________

PART II

NOTE. *All yes and no questions to be answered by code 1 for yes, 0 for no.*

1 Have you ever filled out this questionnaire before? Code Answer ________

If yes, when ____________

2 Do you wear lens correction Code Answer ________

b Do you have an eye muscle defect? Code Answer ________

3 Do you have hearing defect?

	Right Ear	Left Ear	Both Ears	None
Code	*R*	*L*	*B*	*0*

Describe ____________ Code Answer ________

4 Do you wear hearing aid? Code Answer ________

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Indicate by the proper code taken from the appropriate table below and for each type of motion listed. I, the *number of exposures* and II, the *intensity of the symptoms* experienced during your youth and adult life.

I. *Exposure* — None: Code Leave Blank; 1–10: *1*; 10–25: *2*; 25–50: *3*; 50–100: *4*; > 100: *5*

II. *Intensity of symptoms* — Code: None, Leave Blank; Very mild *1*; *2*; *3*; *4*; Very severe *5*

	I. Exposure	General Discomfort	Nausea (Code 1–4)	Vomited or Retched (Code 5)	Stomach awareness or discomfort	Increased salivation	Giddiness, dizziness or vertigo	Drowsiness	Cold sweating	Increased body warmth (not from exercise)	Headache	Pallor	
Swing or other gymnastic equipment													
Carnival devices													5
Cinerama movie													
Airplane-turbulence													5
Airplane-acrobatics													5
Airplane-Zero g maneuvers													5

2a Number of hours in multi-engine aircraft. (Circle one or more of the following: Passenger Crew Military Commercial.)

Hours	None	Less than 10	10–50	50–200	200–1 000	More than 1 000
Code	*0*	*1*	*2*	*3*	*4*	*5*

Code Answer X ________

b Number of hours in single-engine aircraft. (Circle one or more of the following: Passenger Crew Military Commercial.)

Hours	None	Less than 10	10–50	50–200	200–1 000	More than 1 000
Code	*0*	*1*	*2*	*3*	*4*	*5*

Code Answer X ________

From your flying experience where unusual motion is felt would you say that you.

	No flying experience	Never get sick	Rarely get sick	Sometimes
Code	Leave Blank	*0*	*1*	*2*

	Frequently	Most of the time	Always
Code	*3*	*4*	*5*

Code Answer S ________

3 How many exposures have you had to (moderate to violent) wave motion in a ship or boat?

Exposure	None	1–5	5–10	10–50	50–100	Over 100
Code	*0*	*1*		*3*	*4*	*5*

Code Answer X ________

b From your experience at sea what is your (a) average level and (b) maximum level of symptoms when there is moderate to violent motion

Average intensity of symptoms

	None					Severe (Vomited)
Code	*0*	*1*	*2*	*3*	*4*	*5*

a. Code Answer (Average): S ________

b Code Answer (Maximum): S ________

4 In general, how susceptible to motion sickness are you

	Not at all	Minimally	Slightly	Moderately	Very	Extremely
Code	*0*	*1*		*3*	*4*	*5*

Code Answer S ________

5 Experience with Scuba Diving. Yes–No Code Answer ______

a. Number of Exposures

None Less Than 10 10–50 50–200 200–500 More than 500

0 *1* *2* *3* *4* *5* Code Answer ______

b. Average Depth ______ Maximum Depth ______

c. Dives made in the past week ______

Depth (feet) ______ Duration (Hrs., Min) ______

Dates ______

6. Experience with high g force.

Times exposed to 3–5 g None 1–5 5–10 10–20 20–30 Over 30

Code *0* *1* *2* *3* *4* *5*

Code Answer ______

No of times exposed to greater than 5 g: None 1 3 3–5 5–10 10–20 Over 20

Code *0* *1* *2* *3* *4* *5*

Maximum g exposure ______ Code Answer ______

7 Number of years experience with firearms.

None 1 yr 1–3 yrs. 3–5 yrs. 5–10 yrs. More than 10 yrs.

Code *0* *1* *2* *3* *4* *5* Code Answer ______

Trigger with right (Code *R*) or left (Code *L*) hand? Code Answer ______

Average number of pistol rounds fired per year ______

Average number of rifle rounds fired per year ______

8 Exposure to high intensity noise?
If YES (*Code 1*), describe. Code Answer ______

9 Have you ever had an ear illness or any injury or illness which was accompanied by dizziness and/or nausea? Code Answer ______

Approximate Date(s) ______

10. In the past 8 weeks have you been nauseated FOR ANY REASON?
If YES (Code *1*), explain. Code Answer ______

a. In the past you

Code

0 — Were never nauseated in youth or adult life
1 — Could never vomit when nauseated
2 — Would retch and finally vomit
3 — Vomited easily Code Answer ______

11 Have you ever had a serious head injury? Code Answer ______
If YES

a When? ______

b. Describe ______

12 Almost all pilots have had one or more experiences with vertigo and/or disorientation.
How many have you had?

None Less than five Five to ten More than ten

Code *0* *1* *2* *3* Code Answer ______

13 Most people experience faintness or dizziness two or three times a year which is not the result of motion.
During the past year have you experienced faintness or dizziness?

Never Less than this The same as this More than this

Code *0* *1* *2* *3* Code Answer ______

14 Have you been exposed to any rotational test within the past 48 hours
If yes, describe. Code Answer ______

PART III

Motion sickness susceptibility is revealed by a wide variety of subjective symptoms and objective signs resulting from various types of motion and may be experienced over a wide range of severity Common symptoms are discomfort lack of appetite, nausea dizziness and drowsiness, common signs are pallor sweating, increased salivation and vomiting. Most persons recall accurately severe symptoms but not mild symptoms which, even when experienced, may not have been attributed to motion In identifying your motion sickness susceptibility you should relate the acute onset of symptoms to the onset of motion. Symptoms of fear and anxiety do not qualify as indicators of susceptibility to actual motion.

Indicate by the proper code taken from the appropriate table below and for each type of motion listed, I, the *number of exposures* and II, the *intensity of the symptoms* experienced during your youth and adult life.

I *Exposure* Code:
None Leave Blank
1–10 *1*
10–25 *2*
25–50 *3*
50–100 *4*
100 *5*

II. *Intensity of symptoms*
Code: None Leave Blank; Very mild *1*; *2*; *3*; *4*; Very severe *5*

	I Exposure	General Discomfort	Nausea (Code 1–4)	Vomited or Retched (Code 5)	Stomach awareness or discomfort	Increased salivation	Giddiness, dizziness or vertigo	Drowsiness	Cold sweating	Increased body warmth (not from exercise)	Headache	Pallor	
Swing or other gymnastic equipment													
Carnival devices													5
Cinerama movie													
Airplane turbulence													5
Airplane-acrobatics													5
Airplane-Zero g" maneuvers													5

a. Number of hours in multi-engine aircraft. (Circle one or more of the following: Passenger Crew Military Commercial.)
Hours None Less than 10 10–40 50–200 200–1 000 More than 1 000
Code 0 *1* *2* *3* *4* *5*

Code Answer X ______

b. Number of hours in single-engine aircraft. (Circle one or more of the following: Passenger Crew Military Commercial.)
Hours None Less than 10 10–50 50–200 200–1 000 More than 1 000
Code *0* *1* *2* *3* *4* *5*

Code Answer X ______

From your flying experience where unusual motion is felt would you say that you:
Code: No flying experience Leave Blank; Never get sick *0*; Rarely get sick *1*; Sometimes *2*
Code: Frequently *3*; Most of the time *4*; Always *5*

Code Answer 8 ______

5 How many exposures have you had to (moderate to violent) wave motion in ship or boat?
Exposure None 1–5 5–10 10–40 40–100 Over 100
Code 0 *1* *3* *4* *5*

Code Answer X ______

6 From your experience at sea what is your (a) average level and (b) maximum level of symptoms when there is moderate to violent wave motion.
Average intensity of symptoms.
Code: None *0*; *1*; *2*; *3*; *4*; Severe (Vomited) *5*

a. Code Answer (Average): 5 ______
b. Code Answer (Maximum): 8 ______

4 In general, how susceptible to motion sickness are you
Code: Not at all *0*; Minimally *1*; Slightly; Moderately *3*; Very *4*; Extremely *5*

Code Answer 8 ______

5 Experience with Scuba Diving. Yes–No Code Answer ______

a. Number of Exposures

None Less Than 10 10–50 50–200 200–500 More than 500

0 1 2 3 4 5 Code Answer ______

b Average Depth ______ Maximum Depth ______

c. Dives made in the past week ______

Depth (feet) ______ Duration (Hrs., Min.) ______

Dates ______

6. Experience with high g force.

Times exposed to 3–5 g: None 1–5 5–10 10–20 20–30 Over 30

Code *0 1 2 3 4 5*

Code Answer ______

No. of times exposed to greater than 5 g: None 1–3 3–5 5–10 10–20 Over 20

Code *0 1 2 3 4 5*

Maximum g exposure ______ Code Answer ______

7 *Number of years experience with firearms.*

None 1 yr 1 3 yrs. 3–5 yrs. 5–10 yrs. More than 10 yrs.

Code *0 1 2 3 4 5* Code Answer ______

Trigger with right (Code *R*) or left (Code *L*) hand? Code Answer ______

Average number of pistol rounds fired per year ______

Average number of rifle rounds fired per year ______

8 Exposure to high intensity noise?

If YES (Code 1), describe. Code Answer ______

9 Have you ever had an ear illness or any injury or illness which was accompanied by dizziness and/or nausea Code Answer ______

Approximate Date(s) ______

10. In the past 8 weeks have you been nauseated FOR ANY REASON?

If YES (Code 1) explain. Code Answer ______

a In the past you

Code

0 — Were never nauseated in youth or adult life

1 — Could never vomit when nauseated

2 — Would retch and finally vomit

3 — Vomited easily Code Answer ______

11 Have you ever had a serious head injury? Code Answer ______

If YES

a. When? ______

b. Describe ______

12. Almost all pilots have had one or more experiences with vertigo and/or disorientation.

How many have you had?

None Less than five Five to ten More than ten

Code *0 1 2 3* Code Answer ______

13 Most people experience faintness or dizziness two or three times a year which is not the result of motion.

During the past year have you experienced faintness or dizziness?

Never Less than this The same as this More than this

Code *0 1 3* Code Answer ______

14 Have you been exposed to any rotational test within the past 48 hours

If yes, describe. Code Answer ______

PART III

Motion sickness susceptibility is revealed by a wide variety of subjective symptoms and objective signs resulting from various types of motion and may be experienced over a wide range of severity Common symptoms are discomfort lack of appetite nausea dizziness and drowsiness common signs are *pallor sweating, increased salivation* and vomiting. Most persons recall accurately severe symptoms but not mild symptoms which, even when experienced, may not have been attributed to motion *In identifying your motion sickness susceptibility you should relate the acute onset of symptoms to the onset of motion Symptoms of fear and anxiety* do not qualify as indicators of susceptibility to actual motion

Appendix B

SUBJECT'S PREEXPERIMENTATION QUESTIONNAIRE

Name/Number ____________________ Date __________ Time __________

Have you been well throughout the past week?
YES NO

Are you free of all major health complications? (e.g. heart disease, diabetes, back trouble, etc.)
YES NO

Are you in your usual state of fitness today?
YES NO

If no to one or more of the above questions, specify problem and include severity, time course, where localized, etc.

How much alcohol have you consumed during the past 24 hours? (No. and kinds of drinks)

How much tobacco in past 3 hours
Cigarette( ) Cigar(s) Pipe(s) ful

Have you taken drugs or medicines of any kind in the past 24 hours?
YES NO

If yes, what type? If name of drug( ) is known, please list below
- Analgesic (aspirin) __________
- Sedative or tranquilizer __________
- Anti-motion sickness remedy (Anti-histamine) __________
- Other including ear and eye drop medications __________

How many hours sleep did you get last night? Was this sufficient?
YES NO

How anxious are you regarding your participation in these tests?
NOT MINIMAL MODERATE GREAT VERY GREAT

How many hours since your last meal?

How many cups of fluid have you had in the past 2 hours?

Have you served as a subject in any rotational test within the past 48 hours? If yes, endpoint reached.
YES NO

5a. Have you ever taken part in any activities which involved unusual body rotation (dance, game, etc.)? YES (Code 1) NO (Code 0)

Code Answer ________

b. What were they? ____

c. How severe was the motion?

Very mild				Very severe
1	*2*	*3*	*4*	*5*

Code Answer \ ________

d. What was the average intensity of these symptoms?

Very mild				Very severe
1	*2*	*3*	*4*	*5*

Code Answer \ ________

e. What were your specific symptoms?

____

6. How prone are you to car sickness?

	Not at all	Minimally	Slight	Moderately	Very	Extremely
Code	*0*	*1*	2	*3*	*4*	*5*

Code Answer S ________

7 It is thought that there are two kinds of motion sickness. One starts in the brain (dizziness, sleepiness) and the other one starts in the stomach or intestines (vomiting, nausea) Which would you say was typically most like yours?

	Brain	Stomach
Code	*B*	*S*

Code Answer ________

8a. Identify by code the general level of motion sickness susceptibility of your blood relatives when exposed to substantial motion (at sea, in flight or carnival devices, etc.).

Code.		Blood relative	
Leave Blank —	Unknown	Father	Code Answer ________
0 —	Never gets sick	Grandfather	Code Answer ________
1 —	Rarely gets sick	Grandmother	Code Answer ________
2 —	Sometimes	Mother	Code Answer ________
3 —	Frequently	Grandfather	Code Answer ________
4 —	Most of the Time	Grandmother	Code Answer ________
5 —	Always	Other	Code Answer ________

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Name/Number ______________________ Date ________ Time ________

Have you been well throughout the past week?
YES NO

Are you free of all major health complications? (e.g. heart disease, diabetes, back trouble, etc.)
YES NO

Are you in your usual state of fitness today?
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If no to one or more of the above questions, specify problem and include severity time course, where localized, etc.

How much alcohol have you consumed during the past 24 hours? (No. and kinds of drinks)

How much tobacco in past 3 hours?
Cigarette(s) Cigar( ) Pipe(s) ful

Have you taken drugs or medicine of any kind in the past 24 hours?
YES NO

If yes, what type? If name of drug( ) is known, please list below

- Analgesic (aspirin) ____________
- Sedative or tranquilizer ____________
- Anti-motion sickness remedy (Anti-histamine) ____________
- Other including ear and eye drop medications ____________

How many hours sleep did you get last night? Was this sufficient?
YES NO

How anxious are you regarding your participation in these tests?
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How many hours since your last meal?

How many cups of fluid have you had in the past 2 hours?

Have you served as a subject in any rotational test within the past 48 hours If yes, endpoint reached.
YES NO

5a. Have you ever taken part in any activities which involved unusual body rotation (dance, game, etc.)? YES (Code 1) NO (Code 0)

Code Answer: ______

b. What were they? ______

c. How severe was the motion?

Very mild				Very severe
1	*2*	*3*	*4*	*5*

Code Answer X ______

d. What was the average intensity of these symptoms?

Very mild				Very severe
1	*2*	*3*	*4*	*5*

Code Answer: X ______

e. What were your specific symptoms?

______

6. How prone are you to car sickness?

	Not at all	Minimally	Slight	Moderately	Very	Extremely
Code	*0*	*1*	*2*	*3*	*4*	*5*

Code Answer: S ______

7 It is thought that there are two kinds of motion sickness. One starts in the brain (dizziness, sleepiness) and the other one starts in the stomach or intestines (vomiting, nausea) Which would you say was typically most like yours?

	Brain	Stomach
Code:	*B*	*S*

Code Answer: ______

8a. Identify by code the general level of motion sickness susceptibility of your blood relatives when exposed to substantial motion (at sea, in flight or carnival devices, etc.).

Code		Blood relative	
Leave Blank	— Unknown	Father	Code Answer: ______
0	— Never gets sick	Grandfather	Code Answer: ______
1	— Rarely gets sick	Grandmother	Code Answer: ______
2	— Sometimes	Mother	Code Answer: ______
3	— Frequently	Grandfather	Code Answer: ______
4	— Most of the Time	Grandmother	Code Answer: ______
5	— Always	Other	Code Answer: ______

Appendix C

SHEET FOR SCORING SPECIFIC SIGNS AND SYMPTOMS OF MOTION SICKNESS

Symptom Level	Pt. Val.	Principal Symptoms								RPM CW CCW	
Major Minor Minimal AQS	8 4 2 1	TMP[1] II, III	DIZ[2] II III	HAC[3] II III	DRS III II I	SWT[4] III II I	PAL III II I	SAL[+] III II I	NSA II, III I E. D E. A	Other Symptoms	Malaise Level
Head Movements 5											
10											
15											
20											
25											
30											
35											
40											
45											
50											
55											
60											
65											
70											
75											
80											
85											
90											
95											
100											

Based on criteria of Table I
Subjective warmth/flushing. Dizziness Headache Drowsiness. Cold sweating.
Pallor Salivation increase. Nausea. Epigastric discomfort. 14 Epigastric awareness.

Acta

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SUPPLEMENT 275

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ACTA OTO LARYNGOLOGICA

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UPPSALA 1970

For Carol
Danny and Mike

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CONTENTS

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The pathogenesis of the symptom complex which constitutes Ménière's disease or idiopathic endolymphatic hydrops has remained enigmatic. Recent experimental and clinical observations indicate however that an anatomically and physiologically intact endolymphatic sac and endolymphatic duct are requisite for long term maintenance of normal inner ear fluid physiology.

Endolymphatic hypertension or increased pressure within the labyrinth was theoretically suggested (1) as an underlying cause for the clinical triad described earlier by Ménière (3). However little experimental work relating the role of the endolyphatic sac to increased endolymphatic pressure was attempted until Portmann studied the anatomy and the pathophysiology of the endolymphatic sac in lower animals (4 5 6). From these studies Portmann advanced the concept that endolymphatic hypertension in man was the causative factor in Ménière's disease. This concept was clinically applied (7) when the endolymphatic sac was decompressed surgically for clinical endolymphatic hydrops (the Portmann procedure) (8 9 10 11). At about the same time Guild (12) suggested that endolymphatic hydrops would occur with failure of the pars intermedia (rugose portion) to function (resorb endolymph) further that blockage of the endolymphatic duct itself would result in distention of all parts of the membranous labyrinth. Eleven years later the classic histopathologic descriptions of Hallpike & Cairns (13 14) confirmed many of these hypotheses. Interest in the role of the endolymphatic sac in the pathogenesis of clinical endolymphatic hydrops (Ménière's disease), however languished in the ensuing years.

Long term ablative studies on the endolymphatic sac and endolymphatic duct in the guinea pig (15 16 17) cat (17 18 19 20), chinchilla (17) squirrel monkey (17) and rabbit (20), have refocused attention on the definite but species-specific and time-dependent role of the endolymphatic sac and duct in the experimental production of histologically demonstrable endolymphatic hydrops. These results significantly modified the conclusions drawn from the earlier and relatively *short term* ablative studies in monkeys (21) and cats, (22, 23 24), which had failed to show histologically a significant degree of endolymphatic hydrops. Similar relatively short term ablative studies on rabbits (25 26) failed to demonstrate clinical signs consistent with endolymphatic hydrops.

At about the same time that these long-term experimental investigations were in progress, (15 16, 17 18) there was a resurgence of interest in the surgical management of patients with Ménière's disease, and in the Portmann procedure or its modifications (27–33). In addition, other conservation of hearing procedures which involved drainage or release of an increased volume of endolymph from the endolymphatic sac (34–45) and other forms of decompression surgery were being done (46–54). No surgical procedure constructive or destructive however found widespread acceptance (55–58).

During this renaissance of experimental and surgical interest in the endolymphatic sac, Shambaugh observed (38, 59) at surgery a decreased vascularity ("ischemia") of the endolymphatic sac wall in many idiopathic endolymphatic hydrops patients when compared to patients who had the endolymphatic sac un-

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covered during translabyrinthine removal of an acoustic neurilemmoma. The surgical observations by Shambaugh & Clemis (37 38, 59 60 61) of endolymphatic sac wall ischemia, fibrosis and intraluminal adhesions in patients with Ménière's disease have been further substantiated histopathologically (38 60 62, 63 64)

Shambaugh & Clemis currently favor bony decompression of the endolymphatic sac alone (without opening the sac) as the procedure of choice in patients with an early case of clinical idiopathic endolymphatic hydrops with vertigo intractable to medical management, and in which there is hearing worth preserving. Because of this relative contraindication to opening the endolymphatic sac at surgery additional biopsies of the sac wall by these authors are not likely to be taken in the foreseeable future Even with the statistically significant (see statistical study which follows) but highly selective and small series of six biopsy specimens of clinical idiopathic endolymphatic hydrops, these previously reported histopathologic biopsy reports (60 62 63 64) must still be considered preliminary and in need of confirmation

II Histologic Studies

The aim of this report is to strengthen further the histopathologic observations, already reported (60 62, 63 64) of the distinctly abnormal state of the endolymphatic sac epithelium and subepithelial connective tissue in patients with Ménière's disease by comparing our previous biopsy specimens with a larger "normal" or control series of human endolymphatic sacs dissected after necropsy For the most part, the specimens were sectioned undecalcified (and decalcified only when necessary due to the presence of calculi, dystrophic calcification or subepithelial bone) A more satisfactory examination of the histology is possible with undecalcified, dissected specimens. This approach precludes the artifacts produced by prolonged processing and decalcification especially regarding the status of the lining epithelia, the perisaccular connective tissue, and its vascular and lymphatic supply

The detailed case reports of those patients who underwent biopsy of the lateral wall of the endolymphatic sac at the time of endolymphatic sac decompression and drainage surgery were reported elsewhere (60 62) along with the details of the processing and the most pertinent histologic findings. Fig. 1 shows the epithelial distortion in a biopsy specimen from a patient with Ménière's disease It is presented from a previous publication (62) for comparison of the biopsy material with our series of normals Table I (Appendix) summarizes the pertinent clinical history of those patients biopsied, and correlates this information with surgical, histopathologic and polytomographic observations and postoperative results. The clinical implications and surgical indications in these cases (60 67) will be suggested only when all of the information and observations are correlated. Statistics are used to compare the biopsy and necropsy material to determine the significance of the observations made

MATERIALS AND METHODS

As a source of controls and to emphasize the significant abnormalities noted in the biopsy specimens (60 62) endolymphatic sacs were dissected at necropsy from patients with no recorded history of hearing loss (except one patient with presbycusis) This series consisted of 28 sacs from 16 patients, ranging from neonates to an 86-year-old patient (see Appendix, Table II, for age–sex distribution) The dissected material included the endolymphatic sac from the posterior portion of the petrous bone, the proximal interosseous portion (rugose) and the distal intradural portion with a portion of sigmoid sinus and surrounding dura. The specimens were obtained by the use of a dental drill, middle ear instruments, and a Zeiss operating microscope. They were fixed in 10% neutral buffered formalin, dehydrated in graded series of alcohols, but unlike the biopsies were then embedded in paraffin so that thinner serial sections at 8–10 micra could be obtained. It was hoped that this technique would minimize the distortion of the cellular elements and soft tissues encountered in routine temporal bone histopathologic studies caused by decalcification (65), and the additional difficulties inherent in evaluating thick celloidin sections either from bioptic material or temporal bone studies. The following special stains, in addition to Ehrlich's hematoxylin and eosin, were used to help elucidate histochemical characteristics of the endolymphatic sac: Alcian

blue and PAS (mucopolysaccharide) Masson s trichrome (connective tissue), Van Gieson s (elastic and reticulin fibers) and von Kossa (calcium)

RESULTS

Biopsy specimens

The following is a summary of the findings of the biopsy specimens correlated with the clin ical history observations at surgery and pre operative hypocycloidal polytomography The original biopsy patients could be readily di vided into three groups (see Appendix, Table I) Group I ($n = 2$) consisted of those *patients* with clinical idiopathic endolymphatic hydrops *in which the endolymphatic duct* was de monstrated by hypocycloidal polytomography *on the affected side* despite the histologic changes (Fig 1) in the sac (64) and who clin- ically had had the disease for less than two years Remnants of the endolymphatic sac were histologically present but abnormal This group had a favorable result from sac surgery (relief of vertigo and preservation of hearing)

The second group ($n = 4$) consisted of those patients with idiopathic endolymphatic hydrops in which the endolymphatic duct and/or the endolymphatic sac were not demonstrated by hypocycloidal polytomography on the affected side but were visualized on the contralateral side This group had the disease clinically for more than two years Small remnants of endolymphatic sac epithelium were found in- frequently however when present, they were markedly abnormal and distorted With the ex ception of one patient (Case No 4) this group did not benefit from decompression surgery

The third group ($n = 2$) consisted of one patient with symptoms of endolymphatic hydrops secondary to perilymph fistula (Case No 7) (66 67) and another patient with simi lar complaints secondary to congenital lues (Case No 8) (67–73) In both of these cases a *normal* endolymphatic sac and endolymphatic duct were demonstrated radiographically by polytomography The endolymphatic sac epi thelium of both patients appeared essentially normal Neither patient benefited from surgery a finding which was in agreement with previous observations by one of the authors (G E S, Jr) (37 38 42)

Normal microscopic anatomy of the *endolymphatic sac*

The normal microscopic and descriptive ana- tomy of the endolymphatic sac in man has been *reported* (74–96) *and reviewed in detail* (83 92) These studies in man have been prepared *by routine celloidin embedding and thick sec* tioning except for the studies by Sirala (81) *and Secretan* (82) *in which the special thin* celloidin paraffin sectioning method of von *Fiendt & Saxen* (97 98) was used In all these previously reported studies of normal sac with the exceptions noted some soft tissue (espe cially epithelial distortions) were observed

A most recent histologic report on the hu man endolymphatic duct and sac by Zechner & Altmann (96) described many normal mor phologic variations and revealed some new morphologic detail but in regard to Ménière s disease the epithelial changes were con- sidered less important than the obvious fibro- sis of the perisaccular connective tissue They stated that they were unable to find a peri saccular fibrosis in 190 temporal bones from individuals without Ménière s disease com parable to that found in their cases (temporal bones) with Ménière s disease The epithelial cells were described as varying from normal cuboidal to columnar or even pseudostratified. Occasionally protoplasmic processes of epi thelial cells were noted in the subepithelial connective tissue Epithelial hyperplasia was also noted The noncellular intraluminal con tents of hyalinized masses of bony calculi were mentioned

A description of the various portions of the human endolymphatic duct and endolymphatic sac and a nomenclature was proposed by Bast & Anson (83) *They described a sinus and*

isthmus portion of the endolymphatic (otic) duct and three parts of the endolymphatic (otic) sac: the proximal or rugose portion, the sac proper or enlarged flattened portion, and the distal projection. This terminology rather than the more common terminology of the endolymphatic sac established by Guild (99) for experimental animals (100–105) (pars proximalis, pars intermedius, and pars distalis) will be utilized in this report.

In our series ($n = 28$) of normal endolymphatic sacs removed at necropsy the following observations were made. The epithelium was mainly cuboidal (Fig. 2) but varied from a low cuboidal or simple squamous-like epithelium in the proximal portion and distal projection of the endolymphatic sac (Fig. 3 and 4) to a high cuboidal or columnar type of epithelium in the rugose portion (Fig. 5). There was, however a frequent variation in height of epithelial cells, not attributed to plane of sectioning (Fig. 6 and 7). These variations suggest the possibility of a transitional type of epithelium as is found in the urinary bladder able to expand with changes of intrasaccular pressure and thus consistent with the pressure regulating role attributed mainly to the intradural portion of the endolymphatic sac. Epithelial protoplasmic processes into the subjacent connective tissue were infrequently noted (Fig. 8) as previously described (81 82, 96).

True villi (Fig. 9 and 10) were distinguished from simple rugae or folds (Fig. 11) by the presence of a vascular-lymphatic pedicle within the subepithelial connective tissue of the villus. This distinction is important because of the loss of a vascular-lymphatic pedicle in all biopsy specimens from patients with Ménière's disease. By all anatomic criteria these villi could be involved in fluid transport. The hypothesis that the loss of villi in Ménière's disease may be of etiologic importance should be thoroughly examined. These folds also may play a role in the pressure regulating function along with the transitional-like epithelium. Zechner (96) also described a pseudostratified columnar type of epithelium found in response to inflammation. We did not find classical pseudo tratified epithelium but demonstrated occasional areas of stratified and irregularly oriented epithelium. This type of epithelium was apparently a focal metaplasia, most likely an inflammatory or irritative response to intraluminal concretions and/or detritus. These focal areas of metaplasia appeared to consist of layers of surface cuboidal epithelium with a concomitant change in the orientation of their nuclear axis from perpendicular to horizontal in relation to the basement membrane (Fig. 4 and 13). Desquamated or exfoliated epithelial cells were noted in large aggregates within the endolymphatic sac lumen (Fig. 14). In addition, there were specimens in which epithelial hyperplasia was evident and often multifocal (Fig. 15). Other areas of epithelial proliferation (Fig. 16) appeared similar to the fimbriated bodies of pigeon endolymphatic sac described by Ormerod (106). It is also possible that these represent adenomata of the sac epithelium.

Supranuclear and infranuclear epithelial clear spaces (which may be argued to be artifact) (Fig. 17) appeared frequently but irregularly and were consistent with secretory or transportation vacuoles (Fig. 18). The absence of a continuous PAS positive basement membrane in our materials and others (96 107 108) directly related to the presence of vacuoles or "vesicles" was suggestive of significant pinocytotic movement of fluid according to Gussen (107 108). In addition, the luminal surface of the epithelial cells often showed cytoplasmic globules projecting into the lumen of the sac (Fig. 18). This is often associated with "breaks" in the basement membrane (incomplete PAS positive basement membrane) at the light microscopic level. This suggests significant fluid transport by pinocytosis (107–108) rather than herpetic viral vesiculations as suggested by Lempert et al. (109) and House (110) for other portions of the membranous labyrinth. Unfortunately these findings also are consistent with autolysis and a definitive statement may not yet be made. Normal appearing

blue and PAS (mucopolysaccharide) Masson s trichrome (connective tissue) Van Gieson s (elastic and reticulin fibers) and von Kossa (calcium)

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The following is a summary of the findings of the biopsy specimens correlated with the clinical history observations at surgery and pre operative hypocycloidal polytomography The original biopsy patients could be readily divided into three groups (see Appendix Table I) Group I ($n = 2$) consisted of those patients with clinical idiopathic endolymphatic hydrops in which the endolymphatic duct was demonstrated by hypocycloidal polytomography on the affected side, despite the histologic changes (Fig. 1) in the sac (64) and who clinically had had the disease for less than two years. Remnants of the endolymphatic sac were histologically present, but abnormal This group had a favorable result from sac surgery (relief of vertigo and preservation of hearing)

The second group ($n = 4$) consisted of those patients with idiopathic endolymphatic hydrops in which the endolymphatic duct and/or the endolymphatic sac were not demonstrated by hypocycloidal polytomography on the affected side but were visualized on the contralateral side This group had the disease clinically for more than two years Small remnants of endolymphatic sac epithelium were found infrequently however when present, they were markedly abnormal and distorted With the exception of one patient (Case No 4) this group did not benefit from decompression surgery

The third group (n 2) consisted of one patient with symptoms of endolymphatic hydrops secondary to perilymph fistula (Case No 7) (66 67) and another patient with similar complaints secondary to congenital lues (Case No 8) (67–73) In both of these cases a *normal* endolymphatic sac and endolymphatic duct were demonstrated radiographically by polytomography The endolymphatic sac epithelium of both patients appeared essentially normal Neither patient benefited from surgery a finding which was in agreement with previous observations by one of the authors (G E. S., Jr) (37 38 42)

Normal microscopic anatomy of the endolymphatic sac

The normal microscopic and descriptive anatomy of the endolymphatic sac in man has been reported (74–96) and reviewed in detail (83 92) These studies in man have been prepared by routine celloidin embedding and thick sectioning except for the studies by Siirala (81) and Secretan (82) in which the special thin celloidin paraffin sectioning method of von Flendt & Saxen (97 98) was used In all these previously reported studies of normal sac, with the exceptions noted some soft tissue (especially epithelial distortions) were observed

A most recent histologic report on the human endolymphatic duct and sac by Zechner & Altmann (96) described many normal morphologic variations and revealed some new morphologic detail but in regard to Ménière s disease the epithelial changes were considered less important than the obvious fibrosis of the perisaccular connective tissue They stated that they were unable to find a perisaccular fibrosis in 190 temporal bones from individuals without Ménière s disease comparable to that found in their cases (temporal bones) with Ménière s disease The epithelial cells were described as varying from normal cuboidal to columnar or even pseudostratified Occasionally protoplasmic processes of epithelial cells were noted in the subepithelial connective tissue Epithelial hyperplasia was also noted The noncellular intraluminal contents of hyalinized masses of bony calculi were mentioned

A description of the various portions of the human endolymphatic duct and endolymphatic sac and a nomenclature was proposed by Bast & Anson (83) They described a sinus and



Figures 1–4

1. The endolymphatic sac epithelium from patient with Ménière disease (case 1) shows disorganization and nuclear pyknosis. Many epithelial cells are flattened. Note the giant cell (arrow) and the distinctly dense subepithelial connective tissue. A lumen is macroscopically patent (H & E, 400).

2. Normal cuboidal epithelium. Note an occasional fibroblast-like cell below the basement membrane (H & E, 130).

3. In the distal portion of the endolymphatic sac, epithelial height decreases. Within the lumen is a typical assortment of giant cells and detritus (H & E, 315).

4. Squamous (possibly metaplastic) epithelium often occurs in areas where coarse intraluminal detritus is present (H & E, 1260).

epithelial cells interestingly were present in elderly patients when the distal sac wall was collapsed and the epithelial surfaces were in apposition (Fig. 19) This was true even as far as the distal area of the sigmoid sinus (Fig. 20) This observation seemed quite significant when compared to loss of epithelial integrity in the bioptic material (Fig. 1) No evidence of the distortion and loss of epithelium seen in the six biopsies of Ménière s disease was observed in the necropsy specimens Often, large epithelial cells appeared to be either breaking away from the surface to become free-floating phagocytes secreting mucoprotein or engulfing globules of endolymph (Fig 11 and 21) Occasionally cysts (Fig 22) were found arising from the epithelial surface containing highly protein aceous endolymph (19) or early eosinophilic hyaloid masses (Fig. 23 and 24) This re presents protein and mucopolysaccharides in traluminally There was a distinct continuum of eosinophilic or PAS positive endolymph apparently due to concentration effects, from early hyaloid eosinophilic masses (Fig 23 and 24) to dense eosinophilic masses of endolymph within the complicated canalicular system or apparently isolated side pouches (Fig. 25 and 26) as described by Watzke & Bast (84) This process may involve a continuous gradient and include concretions (Fig 26) calculi (Fig 27) and intraluminal bone (Fig. 28) A related process may account for the subepithelial localiza tion of concretions (Fig 29) and dystrophic calcification (Fig. 30) or even bone (Fig. 31)

There was a significant, but highly variable accumulation within the lumen of the endolymphatic sac of free cells of various types (Fig 3 6 12 14 31 32 33 34) giant cells (Fig. 32, 34) detritus (Fig 35 36 37 38) detached cysts surrounded by epithelium (Fig 39) and cellular aggregates (Fig. 14) Clumps of nucleated cells and red blood cells were also found in the endolymphatic sac of a newborn, consistent with that described by Zechner & Altmann (96) in newborns with tentorial ruptures (Fig. 40)

Statistical study

The range of normal variation in the microscopic anatomy of the endolymphatic sac in man has been demonstrated by the examina tion of our material of dissected endolymphatic sacs which were not subjected to decalcifica tion These data were used to emphasize statistically the markedly abnormal status of the endolymphatic sac wall biopsies taken during the endolymphatic sac decompression and drainage procedures (60 62 63 64) Absence, agenesis, or possibly atrophy of old age (9) of the endolymphatic sac in approximately (maximum) 10% of the temporal bones studied (34) or observed at surgery by House (34) Shambaugh (38, 42, 60 62) and Caw thorne (44) appeared to be a significant observation This can occur without clinical symptoms of endolymphatic hydrops. On the contrary Bast & Anson (84) and Richany et al (93) feel that the endolymphatic sac can al ways be demonstrated histologically

In our statistical study (specimens of endolymphatic sac, $n=28$) using the exact proba bility test for a 2×2 contingency table the observed difference (association between histopathologically abnormal endolymphatic sac biopsy specimens and idiopathic endolymphatic hydrops) in proportions of abnormal endolymphatic sac between the select small biopsy group and the random postmortem group is significant at the 1% level ($p<0.001$) In ad dition, assuming a maximum of 10% occur rence of abnormal sacs in the general popula tion (without idiopathic endolymphatic hy drops) there would be one chance in one mil lion that in a sample of 6 all would be abnormal ($p\leq0.001$) Therefore, although the biopsy sample was small, the pathology appeared to be statistically significant.



Figures 1–4.

1. The endolymphatic sac epithelium from patient with Ménière's disease (case 1) shows disorganization and nuclear pyknosis. Many epithelial cells are flattened. Note the giant cell (arrow) and the distinctly dense subepithelial connective tissue. A lumen is microscopically patent (H & E, ×500).

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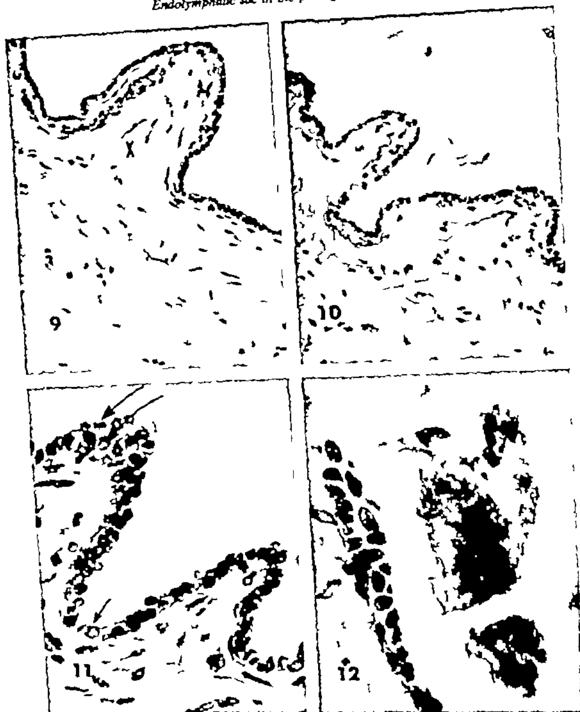


Figure 9–12

9. [illegible] occur in the rugose portion of the endolymphatic sac of neonates. These [illegible] vascular lymphatic pedicle [illegible] than the loose subepithelial connective tissue. Note separate lymphatic-like channel ( ) (H & E, × 280).

10. A ruga or fold does not contain [illegible] vascular-lymphatic complex as found in villi. It is possible that these rugae play an important role in the pressure regulating function of the endolymphatic sac as well as in absorption (H & E, × 280).

11. These large living cells (arrows) morphologically suggest either resorption of endolymph or secretion of mucopolysaccharides (H & E, × 650).

12. Two layers of epithelial cells with vesicular nuclei are noted near condensations in the endolymph. There are desquamated surface cells within the lumen (H & E, × 1170).





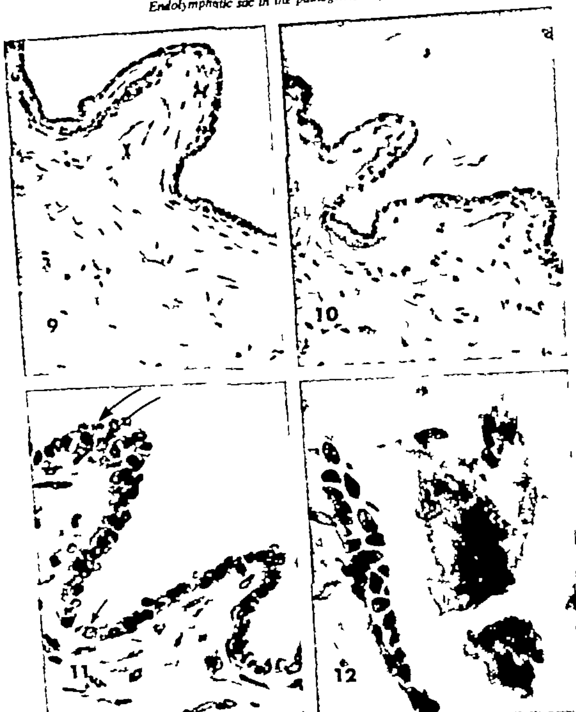

F gu es 5–8

5 Low columnar epithelium frequently demonstrates surface "blebs (arrows) (H & E, 1150).

6 Occasionally the epithelium appears pseudostrat fied or tratified. This, however is consistent morphologically with transitional epithelium (see text). Note the occasional cell with a large acuole (arrow) and surface changes consistent with pinocyto l movement of fluid (arrow) A degenerating cell lies within the lumen (H & E, 1150).

7 Often the epithelium appears multilayered This occurs when there is a surface change in response to irritation by large aggregates of detritus and cal culi. These surface cha ges tend to distort epithelial integrity (H & E, 1150)

8. Subepithelial cytoplasmic extensions occur (ar row) (H E 1150



Figs. 9–12.

9. These [illegible] occur in the rugose portion of the endolymphatic sac of neonates. These have [illegible] vascular lymphatic pedicle [illegible] than the loose subepithelial connective tissue. Note separate lymphatic-like channel (H & E, [illegible]).

10. A ruga or fold does not contain [illegible] vascular lymphatic complex as found in [illegible]. It is possible that these rugae play an important role in the pressure regulating function of the endolymphatic sac as well as in absorption (H & E, 280).

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12. T[illegible] layers of epithelial cells with vesicular nuclei are noted near condensations in the endolymph. There are desquamated surface cells within the lumen (H & E, 1120).



Figure 13 16

13 Below the squamous l ke epithelium are mac rophages and possibly a plasma cell in the perisaccular connective tissue (H & E, 1 60)

14 A large aggregate of desquamated epithelium is present within the lumen (PAS, 315)

15 Epithelial hyperplasia occurs and is often multifocal. These hyperplastic cells ha e a prickl cell appearance This epithelial hyperpla ia wa followed through many serial sect ons (H & F 500)

16. Proximally the sac lumen is patent (arrow) Distally the endolymphatic sac epithelium ends a-ruptly in a tortuous infolded ma s. This may be either an adenoma or a structure nal gous to the fimbriated body reported in the endolymphatic sac of pigeons. (H & F 1 5)









Figs. 17–20.

17. Epithelium with large clear spaces (subepithelial) at one [illegible] occur near epithelium with surface blebs or vesicles (luminal arrow). This is suggestive of either diverse cell types or two different functional states of the same cell (see text) (H & E, ×1150).

18. Similarly two types of epithelial cells can again be seen (arrows) with flattening or extrusion [illegible] epithelial cells (F) into the lumen. This is also suggestive of fluid transport (H & E, ×1150).

19. The distal projection of the endolymphatic sac is lined by low cuboidal epithelium which often lies in apposition without loss of luminal or epithelial integrity (H & E, ×115).

20. Often the distal projection of the endolymphatic sac or tip (arrow) extends to the dural (sigmoid) sinus (SS) with patent uncluttered lumen (L) (H & E, ×45).









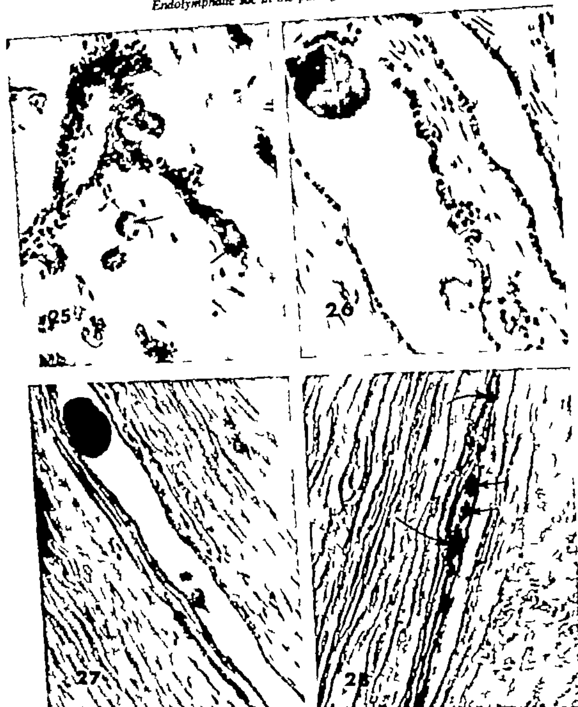
Figure 21 24

1 A large intra-epithelial vacuole of proteinaceous (eosinophilic) material is demonstrated (arrow) (H & E, 1150).

_ Occasionally an epithelial lined cyst (rrow) containing endolymph occurs t the l m l surface Much more frequently however side channel can be mist ken f r yst (see F g 5) (H & E 115)

3 Note large ep thelial cell w th light f amy cytoplasm (rrows) There is an increased cyto- pl mic to uclear ratio There r lso ggreg tes or condensations in the endolymph near these cell (H & E, 1150).

4 Note calcified detritus (black) amid t condensa tions in the endolymph (H & E, 85)



Figures 25–28

25. A multiple canalicular system of side channels of the endolymphatic sac is often filled with dense endolymph (arrows). Note occasional area of hyperplasia (H & E, ×440).

26. This side channel contains condensed endolymph or an early concretion (H & E, ×275).

27. Early intraluminal concretion (C) (H & E, ×110).

28. Intraluminal calcifications, possibly bone (arrows) are present (H & E, ×110).









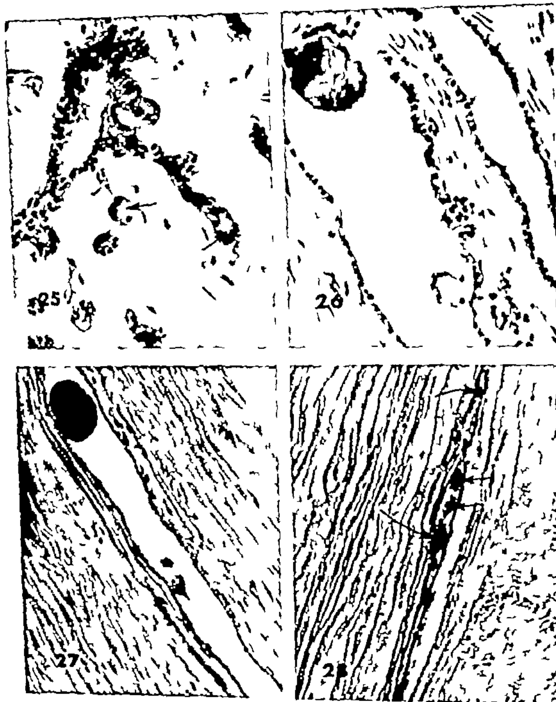
Figures 21 24

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2 Occasionally an epithelial lined cyst (arrow) containing endolymph occurs at the luminal surface. Much more frequently however side cha nels can be mistaken for cysts (see Fig 25). (H & E, × 115)

_3 Note large epithelial cells with light foamy cytoplasm (arrow). There is an increased cytoplasmic to nuclear ratio There are also aggregates or condensations in the endolymph near these cells (H & E, 1150).

4 Note calcified detritus (black) amid t condensations in the endolymph (H & F 85).



Figures 25–28

25. A multiple canalicular system of side channels of the endolymphatic sac is often filled with dense endolymph (arrows). Note occasional area of hyperplasia (H & E, 440).

26. This side channel contains condensed endolymph or an early concretion (H & E, 275).

27. Early intraluminal concretion (*C*) (H & E, 110).

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3 – [illegible]









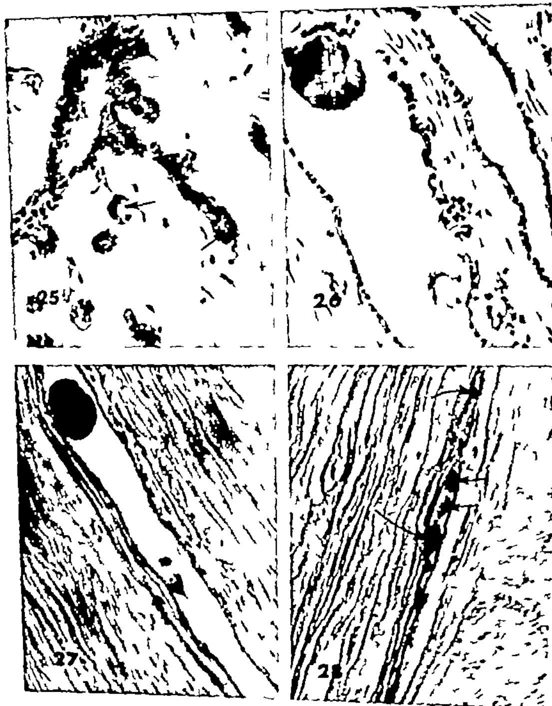
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Figures 25–28

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26. This side channel contains condensed endolymph or an early concretion (H & E, [illegible]).

27. Early intraluminal concretion (H & E, [illegible] 110).

28. Intraluminal calcification, [illegible] (H & E, [illegible]).









Figures 21 4

1 A large intra-epithelial vacuole of proteina ceous *(eosinophilic) material is demonstrated (arrow)* (H & E, 1150).

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Figures 29–32

9 Subepithelial concretion surrounded by a slight inflammatory response It appears to be "breaking through" the epithelial surface (H & E × 280)

30. Subepithelial dystrophic calcification or bone is seen associated with normal cuboidal epithelium without evidence of inflammation. Note the perisaccular connective tissue is loose and well vascularized (H & E × 115).

31 Subepithelial bone is present with metaplastic epithelia apparent only on the abnormal surface No inflammatory response is evident (H & E × 400)

32 Intraluminal granular endolymph is strongly PAS positive Some lining cells appear to be undergoing degeneration (H & E × 1150).



Figures 33–36

33 Subepithelial calcification is present (arrows). Note the variations in types of free cells, degenerating cells, and detritus within the lumen (H & E, 450).

34 Clear areas or globules (arrow) near luminal surface indicate an active metabolic epithelium. Granular giant cells (G) occur within the strongly PAS positive endolymph (H & E, 110).

35 An unusual aggregation of detritus and condensed endolymph is seen in a dilated distal projection of the endolymphatic sac. The proximal point of narrowing can be seen (arrow) (H & E, 40).

36. Multiple concretions are found within the distal portion of a patent endolymphatic sac (H & E, 45).



F gu e 29–32

29 Subepithelial concretion surrounded by a slight inflammatory response. It appears to be "breaking through the epithelial surface (H & E 280).

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III Discussion

Research on the function of the endolymphatic sac has been neglected and requires further elucidation. In a literature review (111) (see Appendix) all of the histologically confirmed cases of noninflammatory (112–114) endolymphatic hydrops in patients with clinical symptoms consistent with Ménière's disease were reviewed. This review (tabulated as Table III, Appendix) (13 14 38, 65 114–145) was directed to the histologic description of the endolymphatic sac and the role (if any) attributed to the sac in the production of hydrops.

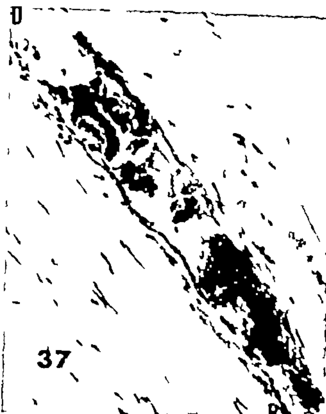
A variety of functions have been attributed over the years to the endolymphatic sac in man and in experimental animals. secretion of endolymph (101 145 146) mucopolysaccharide metabolism (147 148), protein metabolism (106 151 164), resorption of endolymph (12, 81 82, 83 99 149 150 151 153) resorption of metabolic wastes (15) and proteins (148, 154 155) phagocytosis (151), filtration (150, 152), pressure regulation (82, 83 156–163), pinocytosis (104 155) reservoir (100, 165–168), both secrete and absorb endolymph (104), and, a non-functional atavistic structure (74 75 104 169). Which of these functions or malfunctions thereof would predispose a patient to the *maladie du Ménière* cannot be conclusively answered at this time. A conceptual synthesis of the generally accepted functions for endolymphatic sac and the integration of the concepts of longitudinal and radial flow of endolymph is presently in order. This may direct and focus attention on future experimental studies regarding this structure (111)

BASIC CONSIDERATIONS

An abnormal histologic state exists in the endolymphatic sac of patients with idiopathic endolymphatic hydrops or Ménière's disease as demonstrated in six biopsy specimens. This was corroborated by a review of cases of idiopathic endolymphatic hydrops in which the endolymphatic sac was microscopically described (see Appendix) The most commonly reported autopsy finding in the literature was the presence of perisaccular fibrosis or the absence of the loose subepithelial connective tissue. The loose subepithelial connective tissue transmits a vascular lymphatic pedicle and forms the structural basis for the villi. Abnormal or pathologic states of the epithelium lining the endolymphatic sac had not been described consistently as being pathognomonic in medically intractable endolymphatic hydrops before the contributions by Shambaugh et al. (60, 62, 64) Previous investigators were unwilling to trust their observations of epithelial abnormalities because of autolysis and preparation artifact. Since the biopsy material was fixed immediately after excision and not decalcified, the changes in the epithelium are considered significant and not attributable solely to autolysis or postmortem changes.

The epithelium lining the endolymphatic sac is distinctly abnormal in our biopsy material when compared to the necropsy control series. The loss of epithelial integrity is consistent with an old cytopathic inflammatory response, possibly viral in origin, as originally suggested by Shambaugh (60) Other types of inflammatory and infectious processes which may produce similar changes in the endolymphatic sac or in endolymphatic pressure have been reported (73 155 170, 171 172) A marked perisaccular fibrosis of the biopsy specimens was evident when compared to the necropsy series. There was good correlation between pathologic changes of the endolymphatic sac—perisaccular fibrosis, and loss of epithelial integrity—and Ménière's disease.

The endolymphatic sac is considered, there









igures 37–40

37 An the   usual conglomeration of intralu-
inal detritus cells, and calcified material wa
oted The dist l (*D*) portion of the sac i collapsed
e prox mal (*P*) port on is patent (H & E 1 5)
38 A similar amalgam of detritus and cell is
esent at th distal end of the sac from   different
pec men Pre um lly the sac is pate t (H & E   65)
39 An intraluminal y t is urrounded by flat
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aceous material A condensed, acellula lump of
endolymph   lso present (H & E   1300)
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fore, abnormal in patients with Ménière s dis ease and apparently plays a significant ro'e in the pathogenesis of clinical endolymphatic hy drops in man This is particularly true in cases which do not respond to medical therapy and in which symptoms persist for several years. The relatively small group of patients who require surgical intervention for their intractable vertigo may constitute a separate pathologic group from those patients who responded to medical therapy The former group may have an anatomic or histopathologic abnormality of the endolymphatic sac or duct which is per manent and progressive This lesion may over a long period of time produce an irreversible form of endolymphatic hydrops It is possible that patients whose symptoms abate either spontaneously or with medical therapy within a year (173) may fall into other etiologic groups (e g. a vasomotor instability) (174) Such a transitory lesion should not result ini tially in permanent insult to the end organs or the endolymphatic sac In patients diagnosed as having Ménière s disease the percentage demonstrating abnormalities of the endolym phatic sac and/or duct by polytomography was 95% (175 176) This high value indicates however that anatomic abnormalities may be of greater significance than the present study would indicate. Whether these anatomic changes are the primary pathologic focus or the end result of a primary process needs further investigation.

It is speculated that the radial flow of endolymph (132 178–187) which implies localized and rapid formation and resorption of endolymph takes care of the metabolic require ments of the cells lining the scala media, but not those supplied by the Cortilymph (188) Further the longitudinal flow (12 38 81 82 92 99 102 103 149) toward the endolymph atic sac is probably a much slower phenomenon (189) than was originally speculated (12, 99) This may account for the variation in results between the long term and short term ablative studies in various species

The evidence from the biopsy study (60 62) supports the theory that interference with the relatively slow flow of endolymph toward the endolymphatic duct and sac or its resorption places an increased stress on the local re sorptive mechanism which over a long period of time, can result in irreversible clinical and histological endolymphatic hydrops. Local radial circulation maintains local oxygen and metabolic requirements, while longitudinal flow of endolymph aids in bringing detritus to the area where phagocytosis can be most ef fectively carried out and endolymph could be resorbed The vesiculations long noted in the membranous labyrinth are consistent with large movements of fluids (pinocytosis) either locally (radial flow) or to the endolymphatic sac (longitudinal flow) and also autolysis In this theory of phagocytosis, especially the removal of large macromolecules, or the breakdown of large macromolecules into smaller molecules, as suggested by Mygind (190) is inhibited by blockage of lymphatics (evidenced by decrease or absence of loose perisaccular connective tissue which carries the vascular lymphatic complex) then the accumulation of metabolites would attract water resulting in endolymphatic hydrops. Therefore the importance of the endolymphatic sac becomes evident only when its resorptive function is impaired by loss of epithelium in terms of decreased surface area, loss of epithelial integrity decreased blood supply or impaired lymphatic drainage (191)

The endolymphatic sac almost certainly acts as a pressure regulator at least in its distal intradural portion (161) If the patency of the endolymphatic sac lumen is decreased due to inflammation and fibrosis then rapid changes in CSF pressure would remain uncompensated and might result in clinical symptoms

When anatomic abnormalities (perisaccular fibrosis, loss of epithelial integrity or mechanical blockage) metabolic disturbances (electrolyte imbalance and interference with transport mechanisms) or inflammatory processes (viral labyrinthitis) alter the ability of the endolymphatic sac to resorb then the flow of ex

cess endolymph over an extended period of time may lead to clinical and histologic endolymphatic hydrops in man.

CLINICAL CONSIDERATIONS

At decompression surgery (60 61 62) for intractable Ménière's disease, the most consistent findings were *the ischemic appearing sac* wall and the difficulty of locating the lumen of the endolymphatic sac. Clemis & Valvassori (175–176) found by polytomography a markedly narrowed filiform endolymphatic duct (vestibular aqueduct) on the diseased side in a high percentage of patients who had been referred with the clinical diagnosis of Ménière's disease. In contrast, a low percentage was found in patients with other otologic problems. Clemis (175–176) was the first to confirm an obliterated duct at surgery. Obstructions or obliteration of the endolymphatic sac or duct by bone (35 59 96, 117 120, 175 176 192) tumor (116) granulation tissue (116), fibroproliferative arteritis (60, 62, 64) (our case No. 8) and catarrhal swelling of the lining membrane of the (endolymphatic) duct with resultant obstruction of the (intraosseous) endolymphatic duct (70) were other lesions previously reported which may have produced the clinical symptoms of Ménière's disease by means of a mechanical obstruction of the longitudinal flow of endolymph (retention hydrops of Rollin (117)) as speculated by Guild (12).

Clinical observations made at the time of endolymphatic sac drainage and decompression surgery and preoperative polytomographic studies correlated well with our histopathologic observations. All were consistent with a decreased ability of the endolymphatic sac epithelium to resorb endolymph and/or act as a pressure regulator. The following is a preliminary statement of clinical indications and contraindications for endolymphatic sac decompression and drainage surgery based solely on the six biopsy cases in which we were able to make this correlation (see Table I).

Patients with a secondary form of clinical endolymphatic hydrops (Group III) have not generally improved after endolymphatic sac decompression and drainage surgery; therefore in these cases there is no indication for this form of surgery. Patients with primary *idiopathic endolymphatic* hydrops or Ménière's disease, in whom no polytomographic abnormality of the endolymphatic sac and duct was demonstrated and who had had symptoms for only a few years (Group I), generally obtained relief of vertigo and in some cases, preservation of hearing from the surgery. The Group II patients who had polytomographic abnormalities of the endolymphatic sac and duct, generally did not benefit from surgery. The surgical and polytomographic observations on Group II patients correlated well with the histopathologic findings of the biopsy material as follows: (1) the marked fibrosis, (2) decreased distribution of the vascular-lymphatic complex, and (3) absence of any side channels in the more dense lateral aspect of the endolymphatic sac (which was the site of biopsy). This was particularly evident when compared to the residual but abnormal remnants of the side channels of the endolymphatic sac in Group I patients. Clinically Group I patients are considered worthwhile candidates for a simple endolymphatic sac decompression procedure, the earlier the better. In Groups II and III, *this type of surgery has not proved* beneficial and would be contraindicated, based on present knowledge and polytomographic evidence of abnormal endolymphatic sac and duct. In cases of secondary clinical endolymphatic hydrops, this procedure is not indicated. It is recommended, therefore, that early surgical intervention be undertaken only in Group I patients. Polytomography of the endolymphatic sac and duct (175–176) is a necessity in the proper selection of cases for decompression surgery of the endolymphatic sac.

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Acknowledgement

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Appendix

Table I

Histopathologic findings in endolymphatic sac biopsies from patients with various types of clinical endolymphatic hydrops correlated with observations at endolymphatic sac decompression and drainage surgery and with preoperative hypocycloidal polytomographic findings

Case no	Age	Diagnosis	Duration of symptoms (vertigo)	Hypocycloidal polytomography	Surgical observations	Histopathology of endolymphatic sac	Results of surgery
Group I							
1 A K.	39	Ménière s disease (idiopathic endolymphatic hydrops) (hypothyroid)	1 y	Shallow notch for endolymphatic sac and endolymphatic duct	Moderate vascularity adhesions of lumen, I. V nicotinic acid at surgery produced typical skin flush, but no changes in sac wall vessels	Moderate fibrosis, collapsed endolymphatic sac with epithelial sur faces in apposition, distorted epithelium noted loss of subepithelial vascular lymphatic complex (Fig. 1)	Relief of vertigo hearing preserved
2 R. M	58	Ménière s disease (idiopathic endolymphatic hydrops)	20 mo	Prominent groove for endolymphatic sac, endolymphatic duct was also noted	Ischemic wall, lumen difficult to find	Only small remnants of endolymphatic sac, mostly epithelial cell layers in apposition, with loss of epithelial integrity absence of loose subepithelial perisaccular connective tissue	Relief of vertigo hearing stable
Group II							
3 L. M	37	Meniere s disease (atypical id opathic endolymphatic hydrops)	3 y	Unable to identify endolymphatic duct, no indentation for endolymphatic sac on affected side, normal groove and duct on unaffected side	Dilated sac wall vessels, lumen found, no endolymph present, parts of endolymphatic sac wall were adherent	Dense connective tissue, flattened and distorted sac epithelium, microscopic lumen present, dense subepithelial connective tissue, and decreased vascular lympha tic complex present	Improved for 1 year then required labyrinthectomy to relieve the recur rent vertigo
4 C. R.	71	Ménière s disease (idiopathic endolymphatic hydrops)	5 y	Unable to see notch for endolymphatic sac or endolymphatic duct, ppeared normal on unaffected side	Normal vascularity no lumen identified, no endolymph present, obliterated sac	Dense f brotic tissue with decreased vascularity abnormal remnant of epithelium, no suggestion of a lumen	Good results 7 dB hearing improvement, no improvement discrimination

5 I B	71	Ménière disease (idiopathic endolymphatic hydrops)	[illegible]	Endolymphatic sac seen normally bilaterally non-visualization endolymphatic duct on affected side, endolymphatic duct seen on unaffected side	Ischemic sac	Dense avascular connective tissue with absence of any remnants of epithelium or lumen	No significant relief of vertigo or other symptoms
6 M E	72	Ménière disease (idiopathic endolymphatic hydrops)	15 y	Asymmetric appearance of endolymphatic sac groove, unable to see endolymphatic duct on affected side	Sac was white and avascular no definite lumen, no endolymph present	Dense fibrotic and avascular piece of tissue, no lumen, no epithelial remnants, no lymphatics	No improvement
Group III							
7 J O	27	Otosclerosis, stapes surgery with perilymph fistula post operatively symptoms resemble endolymphatic hydrops	1 y	Normal endolymphatic sac and endolymphatic duct, focus of otosclerosis right cochlear capsule and severe bilateral cochlear involvement, spotty areas of remineralization after treatment with NaF	Quite vascular sac and lumen identified with some difficulty walls were somewhat adherent, separated filmy adhesions	Lumen present, relatively loose subepithelial connective tissue, part of the endolymphatic sac was seen with normal patency and eosinophilic and basophilic fluid admixed, normal cuboidal epithelium	No improvement
8 P R.	28	Clinical endolymphatic hydrops secondary to congenital syphilis	6 y	Normal endolymphatic sac and endolymphatic duct, normal otic capsule, no osteitic involvement	Large endolymphatic sac, dilated vessels, thick wall, 2 "tapioca" inclusions removed, could not establish communication between endolymphatic sac and endolymphatic duct	Very vascular connective tissue with patent, but small sac partially occluded, by a fibroproliferative type of arteritis	No improvement
Biopsy control							
9 R. R.	41	Acoustic neurilemmoma	3 y but no vertigo, only unsteadiness	Normal endolymphatic sac and endolymphatic duct, right internal auditory canal much larger than the left internal auditory canal	Vascular sac, patent lumen, no difficulty in identification	Patent lumen, no adhesions, normal cuboidal epithelium	Total removal of tumor via translabyrinthine approach

TABLE II

Distribution of normal endolymphatic sac specimens 28 endolymphatic sacs from 16 random necropsies

Group	Spec. no.	Age	Sex	Race	Ear	Hours postmortem
Premature and fetuses	8 256	28 w	M	N	R	14
	4 780	38 w	M	N	R, L	11
Infants-birth to 1 y	4 787	3 d.	F	W	R, L	28
Young adults 16–25 y	8 166	16 y	M	N	R, L	4
	8 453	18 y	F	W	R, L	11
26–50 y	8 369	27 y	F	W	R	5
	8 161	31 y	F	W	R, L	9
	8 218	42 y	M	W	R L	7
	8 400	49 y	F	W	L	16
51–70 y	4 775	64 y	F	W	R	17
	4 777	65 y	M	W	R, L	20
	4 778	65 y	M	W	R, L	30
	4 788	70 y	M	W	R, L	10
71 y and over	4 781	76 y	M	W	R, L	5
	4 785	86 y	M	W	R, L	4.5
	4 789	86 y	F	W	R, L	-

The following review of the status of the endolymphatic sac in histologically confirmed cases of Ménière's disease (endolymphatic hydrops) (see Table III) is given in chronologic order to enable the reader to follow the conceptual development and changes in interpretation of various authors. Hallpike & Cairns (13, 14) first reported on the histopathologic findings in temporal bones, from their observations in two cases of Ménière's disease. In addition to their classic description of the distention of the cochlear duct and saccule (endolymphatic hydrops) they found significant perisaccular fibrosis which they felt might be a predisposing factor in the development of endolymphatic hydrops. This was the first report of endolymphatic hydrops occurring without associated inflammation. Hallpike & Cairns (13, 14) did a "control" study of 13 temporal bones in which two showed what they considered to be abnormal perisaccular tissue. This "control" study weakened their own observations on the abnormality of the perisaccular connective tissue in Ménière's disease since it could apparently occur as a "normal" variation. With a sample of two ($n = 2$) it would have been risky from a statistical point of view to place too much emphasis on these findings. In both of Hallpike & Cairns cases, there was intractable vertigo for which both patients required surgical intervention (Dandy procedure) and from which both patients expired in the early postoperative period.

Yamakawa (115) reported that in a case of Ménière's disease the lumen of the ductus and saccus endolymphaticus, when compared to the opposite side, was narrowed and filled with thick colloidal masses. Nevertheless, he felt that endolymphatic hypertension was a result of oversecretion by the stria vascularis. He studied another set of temporal bone slides prepared by Dr. Wittmaack with similar observations, but neither case was illustrated by photomicrographs.

Hallpike & Wright (116) found in their one endolymphatic hydrops case an unusual degree of collapse of the lumen with close apposition of the lining epithelium of the endolymphatic sac. A red staining colloid material was found in the sac spaces with a conspicuous absence of perisaccular connective tissue. The epithelium was in close contact with the overlying dura.

Rollin (117) reported the findings in five cases of endolymphatic hydrops. Subepithelial (perisaccular) fibrosis was present in two cases (Cases 1 and 2), two cases (Cases 3 and 5) had obstructive lesions of the endolymphatic sac, and in a final case (Case 4), a congenital agenesis of the sac was reported. In Case 1 the affected (left) side had a marked fibrosis of subepithelial connective tissue with collapsed, adherent, and atrophic epithelium. The lumen was narrow and dark colloidal masses were found within it. Remnants of the lumen contained a large amount of subepithelial pigmentation which was greater in the subdural than in the intraosseous portion of the endolymphatic sac. There was, in addition, some bony (lacunar) erosion. The opposite side was described as having a free lumen with clear endolymph and normal folds of epithelium. Rollin felt both his first and second cases showing perisaccular fibrosis were a "retention" type of hydrops, with a loss of function of the resorptive epithelium, due to inflammatory or to degenerative changes. Rollin's second case had chronic agranulocytosis and myeloblastic leukemia and incidental, bilateral otosclerotic foci. On the more affected side (left) there was a smooth epithelium without folds in the interosseous part, while the intradural portion had definite folds with colloidal

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material. The epithelium, however remained flattened and almost in apposition without side pouches. There were subepithelial venous congestion and fibrosis. On the right side (normal) there was a wide intraosseous lumen with slightly decreased rugae. There were some epithelial fibrosis and pigment present. He did not feel that these sac changes were related to the hematologic problem since they were essentially unilateral and consistent with a loss of function of the endolymphatic sac

Rollin diagnosed his third case clinically as Ménière s disease and pathophysiologically as a retention hydrops" The patient died of metastatic breast carcinoma and, interestingly metastatic carcinoma was present in the lumen of the endolymphatic sac. The m tastatic carcinoma in the endolymphatic sac interrupted the connection of the endolymphatic sac with its distal part on the left side The lumen was filled with albuminous masses. There was a small area of apparently normal rugose epithelium at the junction of endolymphatic sac with the duct. On the right side (normal hearing) a patent lumen was filled with colloidal fluid The epithelium did not have normal folds and the subepithelial tissue was fibrotic Intradurally the endolymphatic sac was flattened and walls were partly adherent Where actual remnants of lumen were present, they contained lumps of colloidal material In this clinically unilateral case, where both endolymphatic sacs were abnormal but different from each other Rollin felt that the metastatic carcinoma caused the hydrops, either from carcinoma metabolites (toxic) or from mechanical blockage

In the fifth case (117) of unilateral retention hydrops, no clinical history was available to Rollin The patient was described as having a normal endolymphatic sac on the right consistent with his age" On the left side, however there was a focus of osteodystrophic bone, which completely blocked the lumen at the point where the endolymphatic duct widens into the endolymphatic sac. Beyond this point, the endolymphatic sac was collapsed and the epithelium was adherent The saccular part of the endolymphatic duct was enlarged. The retention hydrops in this case was attributed to the *obstruction by a* bony closure.

Rollin s fourth case had bilateral endolymphatic hydrops and similar changes in the endolymphatic sac bilaterally This patient had an abnormally shaped head and congenital idiocy Tuberculosis of the kidney lung and larynx were found at necropsy The endolymphatic duct had a dilated lumen The endolymphatic sac had no distinct lumen and there was a flattened and adherent epithelium There was bony erosion on the medial sides. This was considered a complete obliteration of the endolymphatic sac bilaterally Rollin noted enlarged cochlear aqueducts and questioned the influence of the cerebrospinal fluid on the endolymph and perilymph as a possible predisposing factor in endolymphatic hydrops, but he felt that the abnormal endolymphatic sac was related more certainly to the hydrops of endolymph as this would interfere with resorption.

Lindsay (118) noted changes in the endolymphatic sac but made no initial statement as to their significance rather he emphasized vasomotor instability as the cause for the Ménière s disease He described the lining on the affected side as having fewer folds and convolutions than expected. The lumen was patent, but the surrounding connective tissue appeared fairly dense with fewer dilated capillaries than usual The epithelium appeared in good condition The unaffected side showed no definite abnormality

Altmann & Fowler (119) reported two cases (Cases 1 and 3) of asymmetrical perisaccular fibrosis and one case (Case 2) of bilateral fibrosis. They too considered these findings to be insufficiently significant to be impressive" although in their case of *bilateral fibrosis* they felt that perisaccular fibrosis might be "a predisposing factor in the development of endolymphatic hydrops." The first case had a marked fibrosis on the affected side and a

somewhat less pronounced fibrosis on the healthy side. The intermediate dilation (Sinus II) showed relatively few folds and complete absence of the subepithelial layers of well-vascularized connective tissue. The epithelium was flattened. Many of the recesses contained pink staining colloid-like masses. The endolymphatic sac (Sinus III) was well-filled and considered a normal structure. On the opposite side there was a layer of loose connective tissue· otherwise, the perisaccular tissue was fibrotic. The lumen of the endolymphatic sac was patent bilaterally In the second case, a definite fibrosis was present bilaterally· nevertheless, vasomotor changes were felt to be more important than changes in the resorptive mechanism They described a dilated proximal sinus (endolymphatic duct). In the intermediate part, the perisaccular connective tissue was completely absent, and the number of folds was reduced. The lining epithelium was cuboidal. There were "ramified cells around the lumen with coarse brown granules of hemosiderin pigment. The endolymphatic sac (proprius) was absent from the specimen as the dura was stripped at necropsy Similar changes were described on the opposite side, except there were no pigmented cells, but the perisaccular connective tissue was fibrotic.

Altmann & Fowler's third case (119) had fibrosis only on the less affected side. On the affected side, the endolymphatic duct was reported normal. *The intermediate part* showed a moderate number of folds covered with flattened epithelium. The subepithelial layer of loose areolar tissue was present. The recesses contained small colloid-like concretions. On the less affected side, the endolymphatic duct was normal. The intermediate portion showed a complete absence of the loose subepithelial tissue In reviewing and re-evaluating these cases in 1968 Altmann & Zechner (120) stated that marked fibrosis was noted on the diseased side and somewhat less pronounced fibrosis on the healthy side in a patient with unilateral Ménière s disease (Case 1). In a patient with bilateral involvement, a marked fibrosis was present on both sides (Case 2) In a third patient (Case 3) again with *bilateral* involvement, the number of folds in the pars rugosa was reduced, but the loose subepithelial tissue was present on one side but seemed to be absent on the other side

In 1944 Lindsay (121) reported another clinically diagnosed case of Ménière s disease in which the *aqueductus endolymphaticus* showed fewer convolutions in the second portion than are usually present, but the saccus had been mostly torn away with the dura at autopsy The endolymphatic aqueduct showed less perisaccular connective tissue than was sometimes found. Again he doubted that the hydrops could be explained on this basis.

Two years later Lindsay (122) described two more cases. In the first, a ruptured saccule was noted, the only mention of the endolymphatic duct being that a few rugae were present in Sinus II and that the fluid showed an affinity for the eosin stain. In the second case, there was a dilated medial portion of the *aqueductus endolymphaticus* (Sinus II) He felt that the histologic appearance of the epithelium and surrounding connective tissue was normal. He concluded on the basis of these findings and his as yet unpublished monkey experiments (21), that the endolymphatic sac and Sinus II were not essential for maintenance of the normal quantity of endolymph.

After Lindsay's reports (118 121 122) regarding the insignificant role of the endolymphatic sac in the pathogenesis of clinical endolymphatic hydrops in man, despite the histopathologic changes which he himself found, there ensued a loss of interest in the histology and the role of the endolymphatic sac in endolymphatic hydrops, with the single exception of Cawthorne (122) until the renaissance in interest previously described.

Then, in 1947 Arnvig (123) described a long standing case in which the endolymphatic duct and sac were found in only a few sections. Many trabeculae and notches were found, and a homogeneous eosin stain was present. The perisaccular connective tissue was dense and

fibrous On the opposite side, the perisaccular connective tissue appeared normal In the duct, there was a small fibroma which projected half way into the lumen (non-obstructive lesion) Arnvig, like his contemporaries concluded that " it will be hardly justifiable to attach any etiologic significance to the fibroma in the endolymphatic duct. The same applies to the difference in structure of the perisaccular connective tissue on the two sides"

Cawthorne (124) noted bilateral absence of the loose perisaccular connective tissue In addition, the epithelium was backed by dense fibrous connective tissue lining the bony wall He felt that this was an obstructive distention of the endolymph system based on previous measurements of osmotic pressures of labyrinthine fluids (Aldred) This was the last definite and positive interest, expressed in the literature, regarding the histopathology of the endolymphatic sac in the human until Shambaugh (38) studied the slide collection of Lindsay s cases (118 121–122 125 127–129) at the University of Chicago in 1966 and observed vascular changes in the sac wall Despite Cawthorne s reaffirmation of the significance of the perisaccular fibrosis he found, his contemporaries continued to discount similar observations

Brunner (130) reported two cases of endolymphatic hydrops The first case was a patient with neurosyphilis and not Ménière s disease, in which the endolymphatic duct was dilated and contained a granular exudate The epithelium was coarse and edematous The second patient, who was found at autopsy to have a chronic hydrocephalus and an osteitis deformans, had a slightly dilated endolymphatic duct surrounded by a firm connective tissue On the opposite side there was a slight dilatation of the endolymphatic duct at its junction with the saccule Otherwise the duct was normal and, like the sac, was surrounded by firm connective tissue There was an exudate in the endolymphatic duct Brunner concluded regarding these findings, that perisaccular fibrosis was not a tenable etiology for endolymphatic hydrops because it was found in temporal bones without hydrolabyrinth and because of the results of Lindsay s (63) obliteration studies of the endolymphatic sac in monkeys

Nager (130) found perisaccular fibrosis in one patient but did not consider it a typical or regular finding in Ménière s disease. At necropsy this patient was found to have an internal hydrocephalus. The left endolymphatic duct was surrounded by fibrous tissue at the internal aperture. There was a papillary epithelial proliferation of the intraosseous portion. The sac reached far into the intradural part. In the lumen were cellular elements and thickened endolymph. The subepithelial connective tissue of the endolymphatic sac was unchanged On the right side the endolymphatic duct had rugae with high epithelium light perisaccular fibrosis and decreased vessels. There was an early neurinoma of the modiolus.

Day & Lindsay (125) reported findings in a patient who had undergone a diathermy (Day) procedure seven years prior to death, but they described no abnormalities of the endolymphatic sac or any difference between sides No statement was made regarding the role of the endolymphatic sac.

Lempert, et al (109) showed photomicrographs of a case of Ménière s disease which was apparently not reported previously as a case of Ménière s disease, although the bones were studied in 1937–38 by Wolff This case was previously reported as a case of hydrops associated with otosclerosis (126) No significant clinical history was given nor was any statement made regarding the histology or role of the endolymphatic sac. Epithelial vesiculation was noted and felt to be related to a chronic progressive herpetic neuritis of the vestibular labyrinth

Hallpike & Harrison (28, 132) reported a case of a patient who died in the early post operative period following a Portmann procedure. However at autopsy the posterior surface of the dura was stripped therefore no

description of the endolymphatic sac was possible.

Lindsay & Schuthess (128) reported a case of endolymphatic hydrops without any clinical or histologic evidence of vestibular dysfunc tion no description of the endolymphatic sac was included

Lawrence & McCabe (133) described a case in which the patient died of cardiac arrest after a destructive labyrinthectomy They reported a normal endolymphatic duct, but the endolymphatic sac was stripped away with the dura at autopsy

Kristensen (135–136) reported two cases of Ménière s disease. In the first case, a direct opening was found between the saccus and the utricule. No other statement was made regarding the endolymphatic sac or duct in either case. Likewise, Schuknecht et al. (136–138) described three cases in which the description and role of the endolymphatic sac was not given.

Altmann & Kornfeld in 1965 (139) reviewed most of the previously reported histopathologic cases of Ménière s disease and reported an additional three cases. In 1968 Altmann & Zechner (120) re-evaluated their cases previously reported in 1965 (139) and made somewhat different observations regarding the endolymphatic sac. The following is a statement for each case of the 1965 observations, followed immediately by the 1968 re evaluation. Their first case showed slightly dilated proximal endolymphatic duct. There was a coarse brownish pigment (Turnbull positive) found in the macrophages of the per iductal connective tissue. There was no endolymphatic sac in the sections. In 1965 no statement of function was made regarding this case. In 1968, they (1 0) re-evaluated this case (Case 1) and found diminution of rugae and perisaccular fibrosis bilaterally In the second case, the endolymphatic duct showed no folds but was of normal width. Cells containing brownish pigment were found in the subepi thelial tissue The distal part of the duct was slightly dilated. The endolymphatic sac showed no pathological changes. Otosclerosis (red) was noted. In 1968 (120) with unilateral disease, no significant differences were noted between the two sides. The third case was originally re ported as having a normal endolymphatic sac and duct. Bilateral otosclerosis was also noted. In the 1968 (120) re-evaluation they described a marked perisaccular fibrosis and almost complete absence of the rugae on the diseased side. The unaffected side was not available for comparison.

Buchs (140) stated that the endolymphatic sac and duct were normal in a three day old premature infant who had histologic endolymphatic hydrops.

Gussen (108) reported a marked bilateral distention of the saccule, endolymphatic sac and endolymphatic duct in a patient with an "inherited" sensorineural hearing loss but no other clinical symptoms consistent with Ménière s disease. The cochlear duct, utricle and semicircular canals were of normal caliber Numerous vesicles were present in the epithe lium of the saccule, semicircular canals and endolymphatic sac and duct. This vesiculation and basement membrane changes were felt to be consistent with movements of large amounts of fluid across the membranes (pinocytosis) and not related to herpetic vesicles as suggested by Lempert, et al (109)

Lindsay et al. (141) in 1967 reported another case of endolymphatic hydrops with a *clinical history consistent with Ménière s disease* but they did not describe the duct or sac. Lindsay (65) later summarized the observations of 13 temporal bones from 10 patients with idiopathic hydrops in the University of Chicago collection and again made the following general statement regarding changes in the endolymphatic sac: no firm conclusions on perisaccular fibrosis [can be made], partly be cause of variations in the state of soft tissue structures after processing

Schuknecht (142) described three further cases. In the ductus reuniens of the first case, he found a papillary structure covered by the

fibrous. On the opposite side, the perisaccular connective tissue appeared normal In the duct, there was a small fibroma which projected half way into the lumen (non-obstructive le sion) Arnvig, like his contemporaries concluded that " *it will be hardly justifiable to attach any etiologic significance to the* fibroma in the endolymphatic duct. The same applies to the difference in structure of the perisaccular connective tissue on the two sides"

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In the first new case (120) there was fibrosis of the tunica propria of the pars rugosa on the affected (right) side. The sinus of the endolymphatic duct showed a normal width The isthmus was slightly narrower than on the other side The epithelium was normal The proximal portion of the lumen contained homogeneous eosinophilic masses. The peripheral layers of the connective tissue surrounding the distal portion of the isthmus were dense. The pars rugosa of the endolymphatic sac was shorter than on the other side it showed a narrow lumen which was lined with cuboidal epithe lium and contained a small amount of pink staining masses there were only a few folds in the wall The surrounding connective tissue was dense and very poorly vascularized particular ly in the distal portion of the pars rugosa Due to the shortness of the latter the terminal part of the endolymphatic sac extented further up into the bone than on the other side. The rugae were absent the surrounding connective tissue was fibrotic and almost avascular The intradural portion of the sac showed a narrow lu men lined with low cuboidal epithelium Within the surrounding connective tissue circum scribed areas of hyalinization were noted On the left side, the duct and sac showed a wider lumen than on the right (affected) side the folds in the pars rugosa were well-developed the surrounding connective tissue was loose and better vascularized The lumen was empty except in the distal portion of the sac where it contained a small amount of pink fluid but no cells

The second case (120) was most likely a postinflammatory (predominately) serous laby rinthitis. On the affected side the isthmic por tion of the endolymphatic duct was, for a distance of about 3 mm completely obliterated by newly formed web-like bone The proximal sinus of the endolymphatic duct was narrow The *pars rugosa* had been replaced by dense scar tissue A narrow lumen became recogniz able in the interosseous portion of the sac which was lined with cuboidal epithelium The lumen of the intradural portion of the sac was somewhat wider and filled with homogeneous eosinophilic masses The surrounding subepithelial tissue was markedly fibrotic. The opposite (left) side showed the same changes

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9	Rothn 1940 (117)	5 78 ♂ postmortem	No clinical history			Stroke, pneumonia	( ) obstruction in endolymphatic sac by bony closure endolymphatic duct, ( ) collapsed lumen	( ) obstruction by bone (retention hydrops) decreased resorption
10	Lindsay 1942 (118)	1 67 ♂ 26 hrs	15 yrs.	No history	No	Myocardial insufficiency	( ) f brosis, ↓ vascularity (unilaterally on affected side)	No statement
11	Altmann & Fowler 1943 (119)	#1 54 ♂ postmortem	16 yrs.	Yes	Yes	Postoperative intracranial section of VIII CN	(+ + +) fibrosis, (+) epithelium flattened (asymmetrical) f brosis)	(/) Not sufficiently significant to be impressive
1	Altmann & Fowler 1943 (119)	#2 52 ♂ ? postmortem	yrs.	Yes	No	Multiple myeloma	(+ +) fibrosis	(+) perisaccular fibrosis (a predisposing factor)
13	Altmann & Fowler 1943 (119)	#3 79 ♂ ? postmortem	Not stated	Not stated	No	Pneumonia (bilateral)	(+) fibrosis (+) flattened epithelium (+) concretions (+) decreased rugosities (less affected side only)	No statement (note: all cases 119) were re-evaluated in 1968 by Altmann and Zechner—see text) (120)
14	Lindsay 1944 (1 1)	#1 47 ♂ ? postmortem	3–4 yrs.	Yes	Not stated	Subdural hematoma, fracture base skull	(+ +) decreased rugosities (+) fibrosis	(+/−) doubtful relationship
15	Lindsay 1946 (122)	#1 67 ♂ ? postmortem	23 yrs.	Yes	No	Heart failure cancer of caecum	(+) decreased rugosities	Endolymphatic sac and sinus II (−) not essential for maintenance of normal endolymph (based on monkey experiments) (20)
16	Lindsay 1946 (122)	#2 55 ♂ ? postmortem	5 yrs.	No history	No	Myelogenous leukemia	(−) normal	As above
17	Arnvig 1947 (1.3)	#1 76 ♀ 20 hrs.	40 yrs.	Yes (+/)	No	Broncho-pneumonia	(+ +) fibrosis (unilateral), small fibroma on normal side	(−) no etiologic significance to unilateral f brosis

TABLE III

Summary of histopathologic literature pertaining to endolymphatic hydrops

No	Author Year Reference	Case no., Age, Sex Hours postmortem	Duration of clinical symptoms	Vertigo	Was surgery required or contemplated?	Cause of death	Status of endolymphatic sac (histology)	Role of endolymphatic sac (pathogenesis)
1	Hallpike & Cairns 1938 (13 14)	#1 63 ♂ 6 hrs.	3 yrs.	Yes	Yes	Postoperative intracranial section of VIII CN	(+ +) fibrosis bilaterally	(+ +) predisposing factor
2	Hallpike & Cairns 1938 (13 14)	#2 28 ♂ 4 hrs.	4 yrs.	Yes	Yes	Postoperative intracranial section of VIII CN	(+ +) fibrosis	(+ +) predisposing factor
3	Yamakawa 1938 (115)	#1 61 ♂ ? postmortem	2 yrs.	Yes	No	Pneumonia	(+ +) narrowed lumen	(−)No, stria vascularis oversecretion
4	Hallpike & Wright 1940 (116)	#1 29 ♂ 20 hrs.	6 mo	Yes	Contemplated	Secondary hemorrhage postoperative SMR	(+ +) f brosis, (+ +) epithelial apposition	(+ +) substantiated original view (13 14)
5	Rollin 1940 (117)	#1 48 ♂ ? postmortem	19 yrs.	No history given	No	Glomerulo-nephritis and fibrinous pericarditis	(+ +) abnormal epithelium (+ +) fibrosis, ↓lumen- (affected side only)	(+ +) obliteration retention hydrops secondary to decreased re-sorptive function of endolymphatic sac
6	Rollin 1940 (117)	#2 35 ♂ ? postmortem	3 yrs.	Yes	No	Chronic agranulocytosis and myeloblastic leukemia	(+) flattened epithelium (+) fibrosis (greater on affected side)	Secondary over production, in-creased ion concentration, endolymph greater than perilymph
7	Rollin 1940 (117)	#3 47 ? postmortem	At least 4 yrs	Yes	No	Metastatic breast cancer	(+ + +) obstruc tion of endolym-phatic sac lumen by tumor (+) fibrosis, decrea-sed lumen	(+ +) obstruction and/or increased metabolites (toxic)
8	Rollin 1940 (117)	#4 40 ♂ ? postmortem	2 yrs. pain in ear	No history	No	Hydrocephalus (congenital idiocy) TB of lung, kidney and larynx	(+ + +) absent lumen (+ +) epithelial apposition bilateral	(+ +) interference resorption

9	Rollin 1940 (117)	5 78 postmortem	No clinical history			Stroke, pneumonia	( ) obstruction in endolymphatic sac by bony closure endolymphatic duct, ( ) collapsed lumen	( ) obstruction by bone (retention hydrops) decreased resorption
10	Lindsay 1942 (118)	1 67 ♂ 26 hrs	15 yrs	No history	No	Myocardial insufficiency	( ) fibrosis, ↓ vascularity (unilaterally on affected side)	No statement
11	Altmann & Fowler 1943 (119)	#1 54 ♂ ? postmortem	16 yrs	Yes	Yes	Postoperative intracranial section of VIII CN	(+ +) fibrosis, ( ) epithelium flattened (asymmetrical) fibrosis)	(+/−) Not sufficiently significant to be impressive
12	Altmann & Fowler 1943 (119)	#2 52 ♂ ? postmortem	2 yrs	Yes	No	Multiple myeloma	(+) fibrosis	(+) perisaccular fibrosis (a predisposing factor)
13	Altmann & Fowler 1943 (119)	#3 79 ♂ ? postmortem	Not stated	Not stated	No	Pneumonia (bilateral)	(+) fibrosis (+) flattened epithelium (+) concretions (+) decreased rugosities (less affected side only)	No statement (note: all cases 119) were re-evaluated in 1968 by Altmann and Zechner—see text) (120)
14	Lindsay 1944 (121)	#1 47 ♂ ? postmortem	3–4 yrs.	Yes	Not stated	Subdural hematoma fracture base sk ll	(+ +) decreased rugosities (+) fibrosis	(+/−) doubtful relationship
15	Lindsay 1946 (122)	#1 67 ♂ ? postmortem	~3 yrs.	Yes	No	Heart failure cancer of caecum	(+) decreased rugosities	Endolymphatic sac and sinus II (−) not essential for maintenance of normal endolymph (based on monkey experiments) (20)
16	Lindsay 1946 (122)	#2 55 ♂ ? postmortem	5 yrs.	No history	No	Myelogenous leukemia	(−) normal	As above
17	Arnvig 1947 (123)	#1 76 20 hrs.	40 yrs.	Yes (+/−)	No	Broncho-pneumonia	(+ +) fibrosis (unilateral), small fibrosis on normal side	(−) no etiologic significance to unilateral fibrosis

No	Author Year Reference	Case no Age Sex, Hours postmortem	Duration of clinical symptoms	Vertigo	Was surgery required or contemplated	Cause of death	Status of endolymphatic sac (histology)	Role of endolymphatic sac (pathogenesis)
18	Cawthorne 1947 (124)	#1 46 ♂ ? postmortem	4 yrs.	Not stated	Yes (labyrinth-ectomy)	Acute lymphatic leukemia	(+ +) fibrosis (bilaterally)	(+) fibrosis interferes with resorption
19	Brunner 1948 (130)	#1 68 postmortem	2 wks.	Yes	No	Neurosyphilis (not Ménière s)	Endolymphatic duct-granular (+/−) exudate edematous CT	(−) perisaccular fibrosis is not tenable in pathogenesis
20	Brunner 1948 (130)	#2 58 ? postmortem	30 yrs.	Dizziness" (vertigo not stated)	No	Chronic hydrocephalus osteitis deformans pneumonia	( ) fibrosis	As above
21	Nager 1949 (131)	#1 68 ♂ postmortem	14 yrs.	Yes	Yes, Dandy procedure, patient refused	Left carotid occlusion internal hydrocephalus, Burgher s disease	(+ +) fibrosis normal epithelium decreased vessels	(−) perisaccular fibrosis present but not accepted typical or regular findings
22	Day & Lindsay 1949 (1‿5)	#1 56 ♂ 4 hrs.	8 yrs.	Yes	Yes (Dia thermy) premortem 7 yrs.	Cardiac decompensation	Normal	No statement
23	Lemp rt, t al 1952 (109) Wolff (1‿6)	No history given. One case was from the Washington University collection (5178L) prepared by Dorothy Wolff PhD 1937–1938					No statement	(−) perisaccular fibrosis
24	Hallpike & Harrison 1954 (28 132)	#1 50 1 hr	1 mo	Yes	Yes Portmann procedure	Surgical hypotension, expired 4th postoperative day	Posterior sur face of dura stripped no statement	No statement
25	Lindsay & Schuknecht 1958 (128)	#1 66 ♂ 11 hrs.	Not stated	No clinical or histological evidence for vestibular dysfunction	No	Dissecting abdominal aortic aneurysm	(−) not stated	No statement
26	Lawrence & McCabe 1959 (133)	#1 43 ? postmortem	4 yrs.	Yes	Yes (destructive labyrinth-ectomy)	Cardiac arrest	( ) normal endolymphatic duct, endolymphatic sac stripped with dura t autopsy	(−) negative

27	Kristensen 1962 (134)	#1 66 ? postmortem	43 yrs.	Yes	No	Pelvic tumor origin	( ) direct opening between saccus and utricle	No statement
28	Kristensen 1962 (135)	#2 77 postmortem	27 yrs. R 3 yrs. L	No statement of vertigo	No	Pneumonia	No statement	No statement
29	Schuknecht, et al. 1962 (136, 137 138)	#1 52 ♀ 3 hrs.	3½ yrs	Yes	No	Pulmonary embolism after cholecystectomy	N statement	No statement
30	Schuknecht, et al. (136, 137 138)	#2 66 ♂ 3 hrs	10 yrs.	Yes	No	Congestive heart failure	No statement	No statement
31	Schuknecht, et al. 1962 (136, 137 138)	#3 58 ♂ ? postmortem	2 yrs.	Yes	No	Coronary thrombosis	N statement	No statement
32	Altmann & Kornfeld 1965 (139)	#1 54 ♂ ? postmortem	No history hearing loss or dizziness	No	No	Metastatic cancer from ethmoid and maxillary sinus	Coarse brown pigment (+ Turnbull blue) formed in periductal CT no endolymphatic sac in sections, (+) fibrosis bilaterally	N statement
33	Altmann & Kornfeld 1965 (139)	#2 59 ♂ ? postmortem	4 mos.	Yes	No	Metastatic cancer of lung (otosclerosis)	(+) decreased rugosities endolymphatic duct, normal endolymphatic sac, (+/−) no differences	No statement
34	Altmann & Kornfeld 1965 (139)	#3 70 ♂ ? postmortem	Long duration	No statement	No	High blood pressure, intracerebral hemorrhage (otosclerosis)	(−) Endolymphatic sac nd duct normal restatement made in 1967 (120), (+ +) fibrosis unilateral, decreased rugae (+ +) statement 1968 (120)	No statement Note: all three cases (139) ere reevaluated by Altmann and Zechner (120)—see text

No.	Author Year Reference	Case no Age, Sex, Hours postmortem	Duration of clinical symptoms	Vertigo	Was surgery required or contemplated	Cause of death	Status of endolymphatic sac (histology)	Role of endolymphatic sac (pathogenesis)
35	Buchs 1966 (140)	3 day ♂ premature ? postmortem	--	–	–	Respiratory arrest	Endolymphatic sac and duct normal	No statement
36	Gussen 1966 (107)	#1 not stated	Not stated	Inherited sensori-neural hearing loss	No other clinical history		Numerous vesicles in endolymphatic sac and basement membrane, pronounced distention of endolymphatic sac and duct bilaterally	Related to pinocytotic fluid transport, not viral vesiculation
	Shambaugh 1966 (38)	Reviewed the collection of temporal bones with Ménière s disease at University of Chicago					(+ +) decreased vascularity	(+ +) ischemic sac results in impaired fluid resorption
37	Lindsay Kohut & Sciarrha 1967 (141)	#1 67 ♂ ? postmortem	11 yrs.	Yes	No	Myocardial infarction	No description	(−) no statement
	Lindsay 1968 (65 127 129)	Summary of 13 bones, 10 patients in University of Chicago collection with idiopathic hydrops					(+/−) no firm conclusions on perisaccular fibrosis, partly because of the variations in the state of soft tissue preparation	(−) no statement
38	Schuknecht 1968 (142)	#1 65 ♀ ? postmortem	33 yrs.	Yes	No	Cerebral hemorrhage	(+/−) papillary structure in ductus reuniens histologically similar to choroid plexus	(+ +) It seems probable that Ménière s disease is caused by diminished resorptive function of the endolymphatic sac
39	Schuknecht 1968 (142)	#2 53 ♀ ? postmortem	9 yrs.	Yes	No	Cardiac insufficiency	(+/−) no specific statement	As above
40	Schuknecht 1968 (142)	#3 66 ♂ ? postmortem	10 yrs.	Yes	No	Congestive heart diseases	(/−) o specific statement	A above

	Altmann 1967 (143)	Summary of 10 temporal bones from patients with Ménière disease					"In some patients with hydrops, one finds loose perisaccular tissue and on the other hand, in patients without hydrops, marked fibrosis is found"	No apparent connection between appearance of perisaccular tissue and endolymphatic hydrops
41	Altmann & Zechner 1968 (120)	#1 62 postmortem	4 yrs	Yes up to 4 mos. prior to death	No statement	Multiple myeloma and amyloidosis	(+) fibrosis, ( ) decreased vascularity (unilateral on affected side)	(+ +) the fibrosis of the perisaccular tissue on the diseased side favors the assumption that disturbances in the absorption of the endolymph in this area could play a role in producing the hydrops
42	Altmann & Zechner 1968 (120)	#2 67 ? postmortem	Not stated	No statement	No statement	Scleroderma	( ) narrow lumen, ( ) fibrosis (bony obliteration of the isthmic portion of the duct)	Same as above Note: this paper also reviewed the histology of the endolymphatic sac in previously reported cases by Altmann and co-workers (119 139), see text.
43	Black, et al., 1969 (144)	# 62 ♂ ? postmortem	37 yrs.	Not "active"	Stapedectomy for concomitant otosclerosis	Glioblastoma multiforme	Rugose portion and distal sac absent bilaterally vestibular aqueduct abnormally shortened and straight	(+) Positive, but speculative

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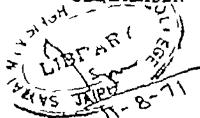
THE BRONCHIAL EPITHELIUM

Nucleic Acid Content in Morphologically Normal, Metaplastic and Neoplastic Bronchial Mucosae

A Microspectrophotometric Study of Biopsy Material

BY

OLE GREISEN



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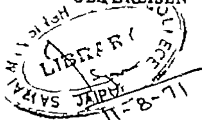
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Denne afhandling er af det lægevidenskabelige fakultet ved Aarhus Universitet antaget til offentligt at forsvares for den medicinske doktorgrad.

Aarhus Universitet den 19 august 1970

G J Bonde
h. a. dec.

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PREFACE

The study presented here was carried out in 1966–1969 during my appointment as Research Assistant in the Institute of Anatomy and the Department of Otolaryngology University of Aarhus.

I owe a very great debt of gratitude to the late Professor Lárus Einarson, M D for the unfailing interest he took in my investigations and for the ideal working conditions he provided for me during my appointment and later when I came as a guest to the Institute of Anatomy I deeply regret that Professor Einarson did not see the completion of the study before his death.

I wish to express my sincere thanks to my chiefs in the Department of Otolaryngology Professor H C. Andersen, M.D and Dr O Elbrønd, for the excellent working conditions which permitted me not only to complete the study but also to preserve the contact with the clinical work in the department.

I am very grateful to Professor Otto Jepsen, M D who personally secured the biopsy specimens and gave me invaluable support and encouragement during the study

It is a pleasure for me to acknowledge my thanks to my brother-in-law Erik Harsaae Professor of Statistics, University of Aarhus, for his unsparing help in the statistical analysis of the material. He developed the formula for the distribution of DNA-synthetizing cells (Chapter 4 Mitoses)

My thanks are also due to M. F Lilsberg, M D., for his aid, especially during the initial phases of the work. With his wide knowledge, Dr Lilsberg greatly contributed to my understanding of the fundamental histochemical aspects of the investigation.

I am indebted to Jørgen Hastrup, M.D of the Institute of Pathology the University Hospital of Aarhus, who kindly and patiently helped me with the evaluation of the histological sections.

I have received much help from Mrs. Inge Lise Walbovd, Secretary Mrs. Ilse Jensen, Laboratory Technician, and Mr Eigil Eriksen, Conservator I wish to express my thanks to them and also to all other members of the staff, who through their co-operation and kindness made it a daily pleasure for me to work in the Institute of Anatomy

A. Rousing, M. T F., has assisted me in the preparation of the English version of the manuscript and in the solution of the technical problems involved. I hereby extend my sincere thanks for his assistance.

Last but not least, my gratitude goes to my wife, Birte for her affectionate support and encouragement during all the phases of this investigation.

Odense September 1970

Ole Greisen

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INTRODUCTION

The cellular content of nucleic acids reflects essential aspects of the function of the cell under normal and pathological conditions.

Most previous studies on the nucleic acid content of the bronchial epithelium are sporadic. It was therefore the purpose of this study systematically to investigate the content of nucleic acids in the nuclei and cytoplasm of the bronchial epithelium under normal and various pathological conditions.

The bronchial epithelium is a very suitabl object for such a study as the epithelial tissu occurs in a number of morphologically well defined states which can contribute to the elucidation of the function of the nucleic acid during changes in the morphology of the epithelium.

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NUCLEIC ACIDS

Chemistry

The knowledge of the nucleic acids began with Miescher's investigations (1897) of a material isolated from pus cells which he called "nuclein

The nucleic acids are built up of chains of nucleotides consisting of a nitrogenous base, a carbohydrate and phosphoric acid.

Both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contain four different nitrogenous bases. Three of them, viz. the purines adenine and guanine and the pyrimidine cytosine are present both in DNA and RNA, whereas the pyrimidine thymine occurs only in DNA, and the pyrimidine uracil only in RNA. This important difference is utilized in tracer studies, in which DNA can be specifically labelled by radioactive thymidine, and RNA by radioactive uracil.

As far as the carbohydrate is concerned, RNA and DNA differ from each other in that RNA contains ribose, while DNA contains deoxyribose. Deoxyribose gives rise to the specific Feulgen reaction of DNA. In the nucleotide, the combination carbohydrate-base forms a nucleoside

The phosphoric acid binds the nucleotides together by attaching the carbohydrates in two adjacent nucleosides by means of an ester bond. These bonds combine a carbon atom (C 3) in one nucleoside and one (C 5) in the next. In this way two of the three acid groups are used to join together two adjacent molecules of carbohydrate. The last acid group is free for combining with basic groups.

While it was formerly thought that the four bases in the nucleic acids occurred in equal amounts and formed tetranucleotides, it is now realized that the proportions of the bases differ in different species of animals, both as far as DNA and RNA are concerned. The smallest unit in the nucleic acids is the mononucleotide (Chargaff 1955)

In DNA the sum of purines is equal to that of pyrimidines. The ratio of the purine adenine to the pyrimidine thymine, and that of the purine guanine to the pyrimidine cytosine are always equal to one. On the basis of these relations and X-ray diffraction techniques, Watson and Crick advanced their theory of the double-helix structure of the DNA molecule

Each molecule of DNA consists of two long chains of polynucleotides forming a double spiral around a central axis. The nucleotides are arranged in a plane at right angles to the chain of polynucleotides, so that the bases are joined to each other by means of hydrogen bonds, and as the distance between the chains of polynucleotides is constant, so that a base of purine in one chain always combines with a base of pyrimidine in the other Owing to the above-mentioned constant relationship between the bases, adenine will always combine with thymine, and cytosine always with guanine. The chains will thus be complementary to each other The sequence of the four bases in a chain of polynucleotides forms the basis for the genetic information. During the doubling of DNA the two chains become detached from each other and each will act as a template for the synthesis of two complementary chains (DeRobertis et al. 1965)

A DNA molecule is very long. The smallest known chromosome is a single-chained DNA molecule, consisting of 5,500 nucleotides. All other isolated molecules of DNA consist of double chains with molecular weights of the

order of 10^9 The whole chromosome in a known bacteriophage is thus one DNA molecule consisting of a continuous double helix comprising about 200 000 pairs of bases and with molecular weights ranging from 1.2×10^8 to 1.6×10^8 (Perutz 1962)

Certain observations suggest that this perfectly regular structure of the DNA molecule cannot in all cases explain the properties of DNA but that there must be certain variations (Bendich & Rosenkranz 1963)

RNA is built up of a polynucleotide chain but as distinct from DNA with uracil and ribose instead of thymine and deoxyribose RNA is present in the cell in different forms.

Transfer or soluble RNA (t or s RNA) exists in a free form in the cytoplasm t RNA is not one substance, but consists of different molecules with molecular weights of the order of 25 000 which corresponds to a length of the polynucleotide chain of 70–80 nucleotides. t RNA contains small amounts of methylated bases in addition to the usual bases in RNA and also small amounts of 5 ribocyluracil nucleotide, in which uracil is bound to C 1 in ribose by means of C 5 instead of N 3 (Perutz 1962) t RNA usually constitutes 10–25 % of the total RNA content of the cell (Hoagland 1960 Amano 1967 b)

As suggested by the term ribosome RNA (r RNA) is found in the ribosomes bound to protein and constitutes about one half of the ribosomes r RNA in E. coli which has been investigated in some detail constitutes about 63 % of the ribosome (Perutz 1962) and consists of at least two different molecules of RNA with molecular weights of 0 56 and 1.12×10^6 respectively It seems to be a common property for all r RNA to have these two different fractions (Spirin 1964) r RNA consists of one long chain of polynucleotides the small r RNA molecule containing about 1,500–2 000 nucleotides, the large molecule 4 000–4,500 nucleotides (Spirin 1964) r RNA constitutes the greater part of the RNA in the cell usually 75–90 % (Hoagland 1960 Amano 1967 b)

Messenger RNA (m RNA) is present in relatively small amounts It constitutes at most 5–8 % of the total content of RNA in the cell Its molecular weight varies widely possibly according to the variations in the size of the proteins which are synthetized in the cell. The molecular weight varies from 2×10^4 to 2×10^6 or even higher (Spirin 1964)

In contrast to the double-chained DNA molecule, r RNA is a flexible single chain of polynucleotides, twisted in compact particles and capable of making reversible changes in form and dimensions and number of intramolecular hydrogenous bonds depending on ionic strength temperature and pH.

The occurrence of double-helix regions has been demonstrated in ribosome and soluble RNA The double-helix structure of RNA is not complete and perfect as in DNA but partial and with defects, i. e. unpaired bases (Doty et al. 1959 a, 1959 b) These helix regions are only relatively short and formed locally by hydrogenous bonds between the bases in the same chain, which is bent back on itself (Perutz 1962 Spirin 1963) The linkage between the bases in the double-helix regions in DNA occurs between complementary bases, so that purines are bound to pyrimidines. The vast majority of the bonds occur between guanine–cytosine and adenine–uracil

Weighty evidence suggests that t RNA is built up of a single chain of polynucleotides which is bent and forms a double helix with itself Foldings may occur in different places, so that the same chain may have several double helix regions, and the axes in these regions will radiate according to the electrostatic forces (Spirin 1964)

A fact which is in favour of the presence of a double-helix structure in the t RNA molecule is that the ratio between the complementary pairs of bases is close to one. This is, however not the case in r RNA (Perutz 1962) and even though double-helix regions have been demonstrated in the r RNA molecule it must mean that they are not nearly as frequent as in the t RNA molecule.

Localization of Nucleic Acids

All DNA in cells from mammals is localized in the cell nucleus (Dounce 1955 Mirsky & Osawa 1961), but in various lower animals and plants DNA has been demonstrated in the cytoplasm (André 1965 Gahan & Chayen 1965). It cannot be determined with certainty whether the greater part or all DNA in the nucleus is bound to protein (Chargaff 1955) The binding occurs primarily to basic proteins, which in higher animals and plants are histones. In spermatozoa, the basic protein is chiefly protamine (Mirsky & Osawa 1961).

After extraction of the nucleohistones, an additional fraction of protein can be isolated from the chromosomes, viz. residual protein, which is a protein with the iso-electric point in the acid area (Mirsky & Ris 1951).

All DNA in the cell nucleus is localized in the chromosomes (Caspersson 1950 Thorell 1955 Mirsky & Osawa 1961) and it is assumed that each chromosome contains a constant amount of DNA (Patau & Swift 1953) Parallelism between the DNA content in the cell nucleus and the number of chromosomes has been reported by many investigators (Swift 1953 Patau & Swift 1953 Richards et al. 1956 Hauschka et al. 1957 Sandritter et al. 1960, Stich et al. 1960 Sandritter Hilwig et al. 1965 Atkin 1967).

After extraction of the nucleohistones, which consist of about 45 % DNA and about 55 % histone protein, about 10 % of the chromosome mass remains. Of this residual chromosome, the residual protein constitutes about 80 % while the RNA content varies between 7.5 and 14 % and the DNA content is only very slight, from 1.5 to 6 % The proportion between the various components of the residual chromosome seems to vary in isolated chromosomes from different tissues (Thorell 1955).

DNA is not just one definite substance, but is found in various forms depending on the proportion between the nitrogenous bases. In all the animal species studied, adenine–thymine predominate, while the content of guanine–cytosine is higher in certain micro-organisms. DNA from different species differ in the composition of nucleotides, whereas DNA from different organs in the same species has the same composition (Chargaff 1955 Mirsky & Osawa 1961).

The DNA content in the cell nucleus is 9–11 % of the dry weight (Pollister 1952 a, Dounce 1955) As first shown by Boivin, Vendrely & Vendrely (1948) and Mirsky & Ris (1949), and later confirmed by many other investigators, the content of DNA per cell nucleus is constant in a given species.

The DNA content is not only constant in different tissues of the organism, with the exception of the germinative cells, but it is also constant and independent even of great physiological influences in the organism. It is thus constant during prolonged fasting and during dietary alterations (Swift 1953 Kurnick 1955 Leslie 1955 Vendrely 1955 Mirsky & Osawa 1961)

An important biological variation in the DNA content occurs during the preparation for mitosis in the form of synthesis and doubling of the DNA content.

The function of DNA in the cell nucleus may thus be said to be the starting point for the synthesis of more DNA in the preparation for mitosis, and as carrier of the genetic properties. DNA is also of importance in the formation of energy-rich phosphate bonds and in the synthesis of adenosinetriphosphate in the cell. This effect is unspecific and can be replaced by other polynucleotides (Allfrey & Mirsky 1957) Finally the presence of DNA is necessary for the synthesis of RNA, and hence for the synthesis of proteins and enzymes in the entire cell.

RNA is present both in the nucleus and in the cytoplasm. In the cell nucleus, about 25 % of the nuclear RNA is found in the nucleolus, the rest in the nuclear sap and in the chromosomes (Amano 1967 a, 1967 b) Most of the RNA content in the cell, about 90 % is found in the cytoplasm, a little is free, but the greater

part is bound in the ribosomes, which in the nerve cells, for example, form the Nissl substance.

After his introduction of the specific staining of the nucleic acids in nerve cells with gallocyanin-chromalum, Einarson (1932) showed that the Nissl substance contained nuclear chromatin substances. He also demonstrated that this substance was formed within the nucleus and diffused through the nuclear membrane (Einarson 1933 1935) and that the Nissl substance consisted of three components viz. a basophilic chromatin substance, a basophilic protein and an acidophilic protein.

That this cytoplasmic basophilic substance, which did not yield the Feulgen reaction, was a nucleic acid (RNA) was later confirmed by several investigators (Caspersson & Schultz 1939 Landström Caspersson & Wolfart 1941 Brachet 1941 Hydén 1943)

The nucleus has an important function in the formation of all types of RNA. Labelling with radioactive substances, which were incorporated during the synthesis of RNA seemed to show that by far the greater part of RNA was formed inside the cell nucleus, but that some formation probably also took place in the cytoplasm (Brachet 1961 Smellie 1963) However other investigators (Taylor 1960 Scholtissek 1966) have shown that all RNA is apparently formed inside the cell nucleus and from here diffuses into the cytoplasm

r RNA is synthetized in the nucleus across the DNA molecule, and probably accumulates in the nucleolus which is formed near the chromocentre (Caspersson 1950) From here it diffuses into the cytoplasm, where it is bound to the proteins in the ribosomes. Electron-microscopic studies have shown that particles of a similar size and structure are present in the nucleus, especially *near the nuclear membrane* and around the nucleolus, and probably derived from the latter (DeRobertis 1956) Chemical *analysis of isolated* nucleoli has revealed a RNA content of the order 2–4 % (Nurnberger et al. 1952, Vincent 1955 Dounce 1955) while Amano (1967 a) reported a RNA content of 13 9 % in nucleoli. A possible source of error in measuring the RNA content in isolated nucleoli is the loss of RNA during isolation. Cytophotometric studies of nucleoli of plant cells at prophase showed a higher content of RNA in the nucleoli (Pollister & Leuchtenberger 1949) but certain objections has been raised against these studies (Dounce 1955)

The ribosomes are nucleoproteins consisting of 40–65 % RNA and 60–35 % protein. The ribosomes are found in all types of cells in all organs without exception (Spirin 1964)

m-RNA is synthetized across the DNA molecule and is complementary to part of one of the chains in the DNA molecule, with the only difference that thymine in DNA is replaced by uracil in m-RNA. It migrates through the nuclear membrane and become attached to the ribosomes. Only bound to them is it able to participate in the synthesis of protein The specificity of the protein which is synthetized depends on the type of m RNA which is attached to the ribosome However r RNA cannot function as a polynucleotide template unless the third type of RNA s-RNA is present in the ribosome One specific s-RNA molecule corresponds to each amino acid. Only when the specific amino acid is attached to s-RNA is it able to participate in the synthesis of proteins which occurs in the ribosomes. In each s-RNA molecule there is a part of the nucleotide chain which is complementary to a part of the polynucleotide chain in m RNA *s RNA is the substance* which translates the code of polynucleotides in m-RNA into that of amino acids, and in this *way it determines the sequence of amino acids* in the protein (Spirin 1964) The synthesis of *proteins thus* occurs in the ribosomes by a complicated interaction between the three different kinds of cytoplasmic RNA. s-RNA constitutes about 25 % of the cytoplasmic RNA and about one third of the RNA content of the cell nucleus (Amano 1967 a, 1967 b)

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members of the same species as in members of different species (Magasanik 1955)

The RNA content per cell nucleus varies considerably in different organs from the same species of animal, as contrasted with the constant content of DNA. Determined by chemical methods, nuclear RNA usually constitutes 7.5–11 % of the total RNA content of the cell (Swift 1953 Mirsky & Osawa 1961) The ratio of RNA to DNA in the cell nucleus varies according to the organ examined and the animal concerned—from 1 3.5 in rat-liver nuclei to 1 17 in calf thymus nuclei (Swift 1953 Dounce 1955)

The RNA content shows considerable fluctuations in cells which undergo changes in mitotic activity differentiation and protein synthesis (Swift 1966 a). During fasting, a considerable reduction in the nuclear content of RNA occurs, while increased mitotic activity tends to increase the content.

Any cell with great synthesis of protein is characterized by a high content of RNA both in the nucleus and in the cytoplasm (Caspersson 1941 Brachet 1941). Thus, this applies to growing cells, and to cells with great activity such as nerve cells and secreting cells (Caspersson 1947)

As shown by Einarson and his coworkers, there is a relation between the functional and pathological activity of nerve cells and their content of nucleic acid (Einarson 1933 1935 1937 1945 1949 a, 1949 b, 1957 Einarson & Lorentzen 1946, Einarson & Krogh 1955 Lorentzen 1950, Bech 1957 Pakkenberg 1960).

The relation between the secretory activity in a cell and its *cytoplasmic basophilic substance* was shown by means of staining with gallocyanin-chromalum in tissues from the pancreas (Oram 1955) the stomach (Weber 1958) and the serous salivary glands (Holtet 1962).

Demonstration of Nucleic Acids

In the demonstration of nucleic acids in situ in histological sections various properties of the nucleic acids are utilized, conditioned by their components of phosphoric acid, carbohydrate and nitrogenous bases.

The demonstration of nucleic acids with basic stains depends on the binding of the stains to the strongly acid groups of phosphate which are liberated by treating the tissues with the stain or fixative (Sandritter 1955 Kurnick 1955 Einarson 1960) The nucleic acids in the cell is linked to protein by phosphate bonds, and the disengagement of these linkages requires a certain time depending on the hydrogen-ion concentration (Lisberg 1962, 1963) Using a number of basic stains with pH levels below 2, Lisberg (1962 1963) found that the staining intensity is proportional to the staining time until the staining intensity reaches a maximum after 24–48 hours. For gallocyanin-chromalum, it has been shown experimentally that the staining intensity in nervous tissue reaches the maximum in the course of 14–16 hours (Pakkenberg 1958). This progressivity of gallocyanin-chromalum staining was emphasized by Einarson right from his earliest works on this staining method (1932, 1933 1934 1935 1951).

In studies on the Nissl substance in nerve cells, Einarson showed that RNA in the cytoplasm was bound to a basic and an acid protein. Mirsky & Ris (1951) found that DNA in the cell nucleus was bound both to basic proteins, histones, and to a strongly acid protein, which they called residual protein. The staining capacity of this acid protein was studied in detail by Lisberg (1963) who found that, both in the cytoplasm and the cell nucleus, it was stained with basic dyes down to a pH of 3–4.6.

A large number of basic stains have been used for the demonstration of nucleic acids, but with few exceptions (Lisberg 1962), no regard was paid to the pH of the staining solution. Specific staining of nucleic acids can be obtained only by stains which are on the acid side of the iso-electric point of the proteins, i. e. at a pH below 2.

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Both DNA and RNA stain with basic dyes.

A certain differentiation can be obtained with methyl green, which stains DNA, and pyronin which stains RNA. The difference in staining of the nucleic acids with methyl green–pyronin is due to differences in the degree of polymerization of DNA and RNA (Kurnick 1955).

With the Feulgen staining DNA can be specifically demonstrated. The specificity is conditioned by the carbohydrate part. The reaction is caused by aldehyde groups which are liberated from deoxyribose in DNA by acid hydrolysis. The reaction can be regarded as a specific qualitative demonstration of DNA. The staining intensity depends on the time of hydrolysis, as a certain liberation and wash-out of DNA occur during the hydrolysis (Kurnick 1955) and it also depends on the fixative used. Finally the tissue may contain other available aldehydes which stain with Schiff's reagent. However, under well-defined experimental conditions and with well-defined hydrolysis times, Feulgen staining does give reproducible values, and it has been used by a large number of investigators for semi-quantitative determinations of the DNA content in cell nuclei.

As shown by Caspersson (1936) the nucleic acids absorb ultraviolet light with an absorption maximum at 2600 A. The specific absorption is due to the heterocyclic nitrogenous purine and pyrimidine bases. Determination of the nucleic acid content by this method requires, however, a complicated and costly equipment.

For relative-quantitative determinations in situ, photometric methods must be used. Here it must be required that the absorbing material is specific for nucleic acids, that the material is suitable for photometry and when stained tissue is used, that the binding of the stain is stoicheiometrical.

Gallocyanin-chromalum

Staining with gallocyanin-chromalum has been used for the determination of the content of nucleic acid in nerve cells under various physiological and pathological conditions (Einarson 1932, 1933, 1935, 1937, 1945, 1949 a, 1949 b, Einarson & Lorentzen 1946, Lorentzen 1950, Einarson & Krogh 1955, Bech 1957, Einarson & Telford 1960). The method has also been used for the determination of the content of nucleic acids in the secretory cells of the pancreas (Oram 1955), the stomach (Weber 1958) and the serous salivary glands (Holtet 1962), as well as in the epithelial cells in the prostate (Jacobsen 1968). Among a large number of other investigators who have employed gallocyanin-chromalum staining may also be mentioned Lagerstedt (1949), Grundmann et al. (1961 b), Dahlgren (1964), Haddad (1968 a), Pakkenberg, and Sandritter and his coworkers.

The theoretical background for the use of gallocyanin-chromalum is briefly as follows.

Gallocyanin, which is an oxazine dye, combines with the metal salt chromalum to a complex compound, a lake, which has a positive charge. This positively charged lake is adsorbed on the negatively charged phosphate groups in the nucleic acids and forms a stable chemical compound with these. Binding to the phosphoric acid in the nucleic acids occurs (Einarson 1951, Lilsberg 1962, 1968). The staining is progressive, i. e. the dye is bound to anions in the tissue until there are no more free negative groups available, after which the staining intensity will not increase. The binding is so stable that the staining is not influenced by alcohol or xylol during the dehydration, or mounting (Einarson 1951, Lilsberg 1962). The staining is not changed by storage for a long time or by exposure to light (Einarson 1934, Oram 1955, Kasten et al. 1962, Sandritter et al. 1963).

The pH in the gallocyanin-chromalum solution is 1.64, i. e. far on the acid side of the iso-electric point of the proteins. At this pH the proteins will have positive charges and will therefore be unable to bind the positively charged dye lake. The only possible explanation of the binding of the stain is linkage of the positive lake cation to negative groups in the strong mineral acids, i. e. the phosphoric acid in the nucleic acids (Lilsberg 1962).

The very weak unspecific co-staining of the tissue which can be seen is probably due to absorption of the basic $(CH_3)_3N$ groups in the dye, the lake sulphate (Einarson 1951) At the low pH the co-staining is without significance, but it increases with higher pH

The strong, deep-blue colour with gallocyanin-chromalum is specific for nucleic acids. The specificity is confirmed by the good agreement which is found between measurements of the nucleic acid content by means of absorption of ultraviolet light and the gallocyanin-chromalum staining (Lagerstedt 1949 Sandritter 1955 Sandritter et al. 1957 Kiefer et al. 1967).

The specificity has also been confirmed by ribonuclease (RNase) experiments. After RNase treatment of nervous tissue it was shown that it was not possible to stain the Nisal substance with gallocyanin-chromalum (Sandritter 1955 Bech 1957 Sandritter et al. 1957 Schümmelfeder et al. 1958 Pakkenberg 1958, Linberg 196.., Goncalves & Haddad (in press), Haddad 1968 a)

Electron microscopic studies have further confirmed the specificity of gallocyanin-chromalum for nucleic acids in yeast cells (Mundkur 1961 1964).

According to the staining theory gallocyanin-chromalum is linked to the strongly acid phosphate groups of the nucleic acids. The proteins are not dissociated, as the only groups which can have a negative charge at this low pH (pH 1 64) are the strong mineral acids. In agreement with this, it can be expected that linkage to other strongly acid groups in the tissues may occur and it has in fact been shown that acid mucopolysaccharides will stain (Sandritter 1955 Schümmelfeder et al. 1958 Linsberg 196.. Haddad 1968 a, b) However it is a characteristic feature that the staining cannot be removed by treatment with RNase and that it is unmistakably metachromatic in the red area, which at the low pH indicates mucin sulphate (Curran 1964) Furthermore, mucin is PAS-positive and can be eliminated a a source of error by its localization and the morphology of the tissue (e. g. in the goblet cells of the bronchial epithelium)

With the above-mentioned reservation, the staining of the nucleic acids with gallocyanin-chromalum is specific, and the blue orthochromatic staining of the nucleic acids is, without reservation, specific for them.

The condition for using a dye for quantitative histochemical determinations is that Beer Lambert s low applies to it, i. e. that the extinction is proportional to the concentration and the thickness of the absorbing substance. That the binding of gallocyanin-chromalum to the nucleic acids is progressive and quantitative was emphasized by Einarson (1947 1951).

Lambert s law which says that the extinction is proportional to the thickness, is valid for nucleic acid-bound gallocyanin-chromalum both in vitro and in histological sections (Diefenbach & Sandritter 1954 Sandritter et al. 1954 Pakkenberg 1958, Sandritter Kiefer & Rick 1963 1966).

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The effect of RNase depends on the conditions under which it acts (the solvent, temperature, pH concentration and time) and first and foremost, on the pre treatment, i. e the fixation of the tissue

The techniques described for the use of RNase vary widely The enzyme is thermostable (Swift 1955) and has been employed by various investigators at temperatures ranging from 20 to 60° most frequently about 37° Depending on the temperature the concentration of the enzyme and the time required vary The pH optimum is within the range from 7 4 to 7 6 (Kaufmann et al. 1951 Jonsson & Lagerstedt 1957 Josefsson & Lagerstedt 1962) RNase is used dissolved in distilled water or in various buffers (Stowell & Zerzoli 1947 Sandritter et al 1957 Goncalves & Haddad 1966) The presence of electrolytes is an advantage in the use of RNase, as a rapid and complete breakdown of RNA will then occur (Greenstein et al. 1947 Markham et al. 1952 Edström 1953)

A notable property of RNA is its solubility During preparation of histological sections a certain loss of RNA from the cells will occur Some fractions are more readily soluble than others The use of fixatives reduces unwanted loss of RNA but some soluble nucleotides are apparently removed (Stowell & Zerzoli 1947 Swift 1966 a) s-RNA is probably removed by most aqueous fixatives, including formalin. Most fixatives preserve protein bound RNA, but strongly acid fixatives can, after fixation for a long time, eliminate some RNA by hydrolysis (Swift 1966 a)

By ultraviolet absorption measurements, Davies (1954) observed loss of nucleic acids both from formalin- and Carnoy fixed nuclei, as well as from formalin-fixed cytoplasm. However a certain loss which may be due to factors other than the fixative cannot be excluded, and no regard was paid to the redistribution after the fixation By chemical determination of RNA in the guinea-pig liver a loss of 20 % was found after fixation in Carnoy s solution for 2 hours (Harbers & Neumann 1955)

In contrast to these studies, Hartleib et al. (1956) did not observe any loss of nucleic acid phosphate by chemical determination after formalin fixation or fixation in Carnoy s solution, nor after further treatment of the tissue. Formalin and most acid fixatives do not cause a significant loss of nucleic acids from the cell (Kurnick 1955)

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Photometry

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It is an advantage to use monochromatic light as the absorption can then be measured at well-defined wave lengths, and it is possible by varying the wave length, to measure the absorption in areas where the extinction is within suitable ranges (Sandritter et al. 1959)

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content of nucleotides from the formalin-fixed tissue, as compared with Carnoy-fixed tissue.

It is likely that not all RNA is decomposed with the same ease by RNase. Cytoplasmic RNA will thus often be removed more easily and *before RNA in the nucleolus* and in the chromosomes (Swift 1966 a, Bonner et al. 1968). Sandritter et al. (1963) showed that a weak staining of the Nissl substance remained in formalin-fixed nervous tissue after treatment with RNase. Similar observations were made by Amano (1962) Haddad (1968 a) and Goncalves & Haddad (in press), whereas Pakkenberg (1958, 1959) showed that all RNA in the Nissl substance was removed by treatment with RNase of formalin-alcohol-fixed brain tissue. The significance of thorough rinsing of formalin-fixed tissue was emphasized by Pakkenberg (1962 a).

RNA in the Nissl substance is removed completely by RNase after fixation *in Carnoy's* solution (Sandritter 1955 Bech 1957 Lilsberg 1962, Haddad 1968 a, Goncalves & Haddad (in press)). However the decomposition of RNA is usually complete after formalin fixation (Stowell & Zerzoli 1947 Lagerstedt 1956, 1957 Sandritter et al. 1957 Swift 1966 a) This has also been shown electron-microscopically by Mundkur (1961 1964)

Jonsson & Lagerstedt (1958) found that by flattening on water of paraffin sections fixed in Carnoy's solution a considerable loss of nucleic acids occurred in the water bath, while no loss was observed in formalin-fixed tissue. Other investigators, too, have found a varying loss of cytoplasmic RNA in Carnoy-fixed tissue in various buffers or in distilled water without RNase (Brachet 1953) while only a very slight loss, or none at all, occurred in formalin-fixed tissue (Stowell & Zerzoli 1947 Lagerstedt 1956, Amano 1962, Goncalves & Haddad 1966)

It is generally assumed that formalin is a suitable fixative for RNA prior to RNase treatment (Kurnick 1955 Pearse 1961 Swift 1966 a).

Photometry

The theoretical and practical basis for microphotometry was elaborated by Caspersson (1936) with the development of ultraviolet microphotometry Photometry in visible light on stained sections is preferred by many investigators on account of a less costly and less complicated equipment which in many respects is fully equal to photometry in ultraviolet light in practical histochemistry (Pollister 1952 b, Swift 1955)

A condition for photometric measurements is that Beer Lambert's law applies, i. e., that the extinction is proportional to the concentration and thickness of the absorbing substance, and in photometry in visible light, that the dye combines stoichelometrically with the substance to be measured. As already mentioned, these conditions are both fulfilled for gallocyanin-chromalum.

The principle of microphotometry is simple. There are certain sources of error both in the apparatus and in the object, but usually errors in the apparatus does not play any significant role (Sandritter 1966)

It is an advantage to use monochromatic light as the absorption can then be measured at well-defined wave lengths, and it is possible by varying the wave length, to measure the absorption in areas where the extinction is within suitable ranges (Sandritter et al. 1959)

A great numeric aperture of the objective should preferably be used, so that the greater part of the transmitted light from the object is included and the *unspecific loss of light is reduced* (Swift 1953 Sandritter 1966) On account of the thickness of the histological sections it is also a *distinct advantage* to use a great numeric aperture *of the objective*.

The numeric aperture of the condensor is not of as great significance as was previously assumed (Sandritter 1966) The condensor aperture *should* be smaller than that of the objective. Davies & Walker (1953) and Sandritter et al. (1959) recommended a condensor aperture of [illegible]

objective A too small numeric aperture of the condensor may cause an unspecific loss of light, while a too great numeric aperture causes an increase in scattered light in the measuring area (Swift 1953) However a great numeric aperture also has an opposite effect, as the extinction on account of the longer oblique path of the light will increase (King & Roe 1953) Generally a condensor aperture of the order of 0 3 is recommended (Swift 1953 Sandritter 1966) but Ris & Mirsky (1950) found only very small changes by varying the condensor aperture from 0 25 to 0 85

Flare light, the Schwarzschild-Villiger effect, is a source of error which increases when the condensor aperture is too great, and when the illuminated area is too large as compared with the measuring area. The error tends to give too low values of the extinction, and increases with increasing extinction of the substance studied (Davies & Walker 1953 Richards 1966) This source of error is of significance only at high extinctions (Swift & Rasch 1956 Leuchtenberger 1958 Müller 1965 Sandritter 1966)

It is of importance for the measuring accuracy that the extinctions are within the range from 0 3 to 0 7 as the photometer is most accurate in this range (Lison 1950 Glick et al 1951 Davies & Walker 1953) Lison (1950) calculated the effective error of extinction measurements at an instrumental error of 0 5 % he found that the error becomes great when the percentage of transmission is greater than 80 or less than 5 i e at extinctions below 0 1 or above 1.3

The possibilities of errors in the object are greater Errors may be due to scattering, the *distribution of the absorbing substance* and the dimensions of the object (cell dimensions, size of the measuring area, thickness of the section)

Light scattering is no major problem in photometry on fixed and stained material in visible light. The scattering is *greater at short wave* lengths, and is a greater problem in photometry in ultraviolet light (Swift & Rasch 1956, Bammer 1960 Sandritter 1966) If the illuminated area is too large, there will be more *scattered light in the area, and it is therefore* an advantage to use a small illuminated area for the measurements (King & Roe 1953 Swift & Rasch 1956 Sandritter Schiemer et al 1958, Sandritter et al. 1959 Pakkenberg 1960) The percentage error increases with higher extinctions of the absorbing substance, for which reason it will be an advantage to measure away from the absorption maximum at high extinctions (Swift 1953 Swift & Rasch 1956)

The importance of the distributional error *caused by a heterogeneous distribution of the* absorbing material was critically assessed on the basis of theoretical calculations by Glick et al (1951) The error was also calculated by Caspersson et al (1957) and by Leuchtenberger (1958)

The distributional error will be greater with a coarser distribution of the absorbing particles The error can be reduced by integrating numerous measurements of a small measuring area (scanning) or by the two-wave method *(Patau 1952 Ornstein 1952) The error can,* however be shown empirically to be only of slight importance (Swift 1953 Lodin et al 1965) By comparative measurements on small and larger areas it can be shown that the distributional error in larger areas is negligible (Swift 1953) By control with the two-wave method it has been shown that the distributional error is of minor importance (Swift & Rasch 1956 Sandritter et al 1959) Similar observations were made by Ris & Mirsky (1950) and Sandritter et al (1960) demonstrated that the distributional error in normal gallocyanin-chromalum-stained nuclei is of the order of 1 % In absorption measurements by the "plug method" in which a measuring area of $^1/_4$–$^3/_4$ of the nucleus diameter is used, no essential error is introduced (Swift 1953 Swift & Rasch 1956) It may be shown mathematically that the error depends on the size of the *plug. With a very small central plug* the error is completely eliminated When the plug comprises the whole nucleus, the error is $^1/_{12}$ When

the size of the plug is $^3/_4$ or $^4/_5$ of the area of the nucleus, the error is only $^1/_{45}$ (Roels 1965)

The fixative is important for the distribution of the absorbing particles. Some fixatives give a coarser clumping than others. The distribution of chromatin is thus more homogeneous after fixation in formalin than in Carnoy's solution (Swift 1950 Pollister et al. 1951 Nurnberger et al. 1952, Davies 1954) The homogeneous distribution of the chromatin after formalin fixation was also emphasized by Kurnick (1955) By comparing nuclei fixed in formalin and nuclei fixed in isopropyl alcohol and a mixture of methanol, formalin and glacial acetic acid, *Böhm (1968) found* that the nuclei fixed in formalin were smaller and darker in colour with a distribution of chromatin which could hardly be differentiated, than after fixation with the other agents. This gives rise to a *smaller* distributional error but, on the other hand, also to extinctions which are too high to be optimal for absorption measurements. The distributional error increases with increasing extinctions (Garcia 1962 b), but can be reduced by measuring away from the absorption maximum where the extinction is greatest (Leuchtenberger 1958)

A special type of distributional error may occur in crystalloid structures owing to dichroism, where the absorbing molecules are orientated so that the absorption is greater in one plane than in another (Swift & Rasch 1956) However in most biological materials it possibly does not occur in sufficient degree to disturb photometric measurements, especially not in photometry on fixed and stained material in visible light, whereas the problem is greater with absorption measurements in ultraviolet light (Swift 1953)

A relative measure of the absorbing substance in a nucleus in measurements *by the* "plug method" can be calculated as the product of the extinction and the area of the absorbing nucleus. Only when the nucleus is a perfectly regular sphere is the *formula* absolutely correct. In biological materials, however practically no nuclei are of this regular shape, but more or less ellipsoid (Palkovits & Fischer 1968) If the asymmetry is not too great, the content of a substance as expressed by the area multiplied by the extinction may nevertheless be used in relative measurements in practical histochemistry (Ris & Mirsky 1950, Swift 1950, Sandritter et al. 1959 Müller 1960 Lison 1960, Jobst & Sandritter 1961 Allen 1962 Sandritter et al. 1963)

The area of the nucleus can be determined by measuring the diameters of the nuclei by means of an eyepiece micrometer (Kracht & Spaethe 1955 Swift 1950 Swift & Rasch 1956, Leuchtenberger 1958, Bader et al. 1960 Allen 1962, Pakkenberg & Vrå-Jensen 1964 Sod Moriah et al. 1968 Sachs 1968, Jacobsen 1968), even though planimetry must be regarded as a more accurate, but also a more time consuming procedure. By measuring the size of nuclei with eyepiece *rings*, *Sandritter et al.* (1960) found an error of 4–6 % as compared with planimetric measurements. Grundmann et al. (1961 a) calculated the area on the basis of the diameters *of* the nuclei in preference to planimetry as only a slightly better accuracy was obtained by the latter method. The measuring error for fixed diameters was very slight with a *coefficient* of variation of 3 % while, with new adjustments, it was 6 % (Garcia 1962 b) When a certain amount of practice and experience has been acquired, it is possible to obtain a reasonable accuracy and reproducibility by means of eyepiece micrometers *(Leuchtenberger* 1958, Jacobsen 1968)

In measuring extinctions in nuclei *it is of* importance that there are no parts of other nuclei in the measuring field. This can be avoided by careful focusing and by the use of great magnification and an objective with great resolution. By careful focusing it must, as far as possible, be ensured that the nucleus to be *measured is not cut, but* this may be difficult to assess with certainty (Sandritter & Pilny 1966)

Slight variations in the thickness of the sections is only *of minor importance in the estima-*

objective A too small numeric aperture of the condensor may cause an unspecific loss of light, while a too great numeric aperture causes an increase in scattered light in the measuring area (Swift 1953) However a great numeric aperture also has an opposite effect, as the extinction on account of the longer oblique path of the light will increase (King & Roe 1953) Generally a condensor aperture of the order of 0 3 is recommended (Swift 1953 Sandritter 1966) but Ris & Mirsky (1950) found only very small changes by varying the condensor aperture from 0 25 to 0 85

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MATERIAL AND METHODS

The material studied consisted of bronchial biopsy specimens from the Department of Oto-laryngology the University Hospital, Arhus. The specimens composing the material of mor phologically normal mucosae were secured during the period from October 1966 to June 1967 The patients were referred for broncho-scopy and possibly biopsy on suspicion of a pathological condition of the bronchial system. All biopsy specimens were taken under the same conditions, during the morning when the patients had been fasting from the evening before, and all patients were in general anaesthesia. The specimens were taken from the carina of the middle-lobe bronchus on the right side or in a few cases where this was not possible, from the carina between the upper and lower-lobe bronchus on the left side. All biopsies were performed by the same operator The specimen was removed from the forceps in physiological saline and immediately placed in 4 % formalin water neutralized by pulverized calcium carbonate. The advantage of using these small fragments of tissue is that imme diate fixation is obtained in the formalin, which has a high power of penetration and preserves the morphology of the epithelium (Wolman 1955 Gersh 1959 Swift 1966 a)

The specimens were fixed for 24 hours and then rinsed in tap water for another 24 hours. On the basis of experiments with human biopsy specimens the suitable time for the further treatment of the tissue was found to be 30 minutes 70 % alcohol (3 changes) 30 minutes 96 % alcohol (3 changes) 30 minutes 99 % alcohol (3 changes) After this the spe-cimens were placed in toluol until they became clear i.e for a couple of hours. They were then embedded in paraffin, 3 × 10 minutes at the melting point of the paraffin. The block was cut by a Leitz sledge microtome with an adjustment of the thickness of sections at 7 µm. The sections were flattened on water at 40 degrees in the shortest possible time, placed on a slide and deparaffinized in xylol, rehydrated in alcohol, rinsed in distilled water and stained with gallocyanin-chromalum for 48 hours.

The gallocyanin-chromalum solution is pre-pared as follows (Einarson 1951)· 10 g chrom alum is dissolved in 200 ml distilled water The solution is brought to the boil just before the boiling point is reached 0.3 g gallocyanin is added ("Chroma gallocyanin, D Grübler & Co., Stuttgart) The mixture is boiled for 5 minutes, and after cooling to room tempera-ture the solution is filtered, and distilled water is added through the filter until a volume of 200 ml is reached again. The dye solution was used to up 14 days after preparation.

After staining in a dark place for 48 hours the sections were rinsed 4 times in distilled water and after treatment with alcohol and xylol they were mounted in D P X. mountant (Kirk, Patrick & Lendrum, Edward Gurr Ltd., London) which has a refractive index of 1.515 The enzyme-treated sections were treated with RNase in a concentration of 1 mg per ml of Sorensen s phosphate buffer at pH 7 6 for two hours at 37 C (Ribonuclease-A Sigma, St. Louis, Missouri, U S. A.) The sections were then mounted on slides (Gold-Seal microslides, Clay Adams Inc., New York) of a thickness of 1.0 mm. The thickness of the cover glasses was 0 13 mm.

From each biopsy specimen, some sections were stained with haematoxylin-eosin for sur vey microscopy and with PAS. The thickness

tion and comparison of relative-quantitative determinations of the absorption by the cell *nuclei* All particles in the light conus participate in the light absorption, but to different degrees, so that the importance is inversely *related to the distance of the particle from the* plane of focus (Einarson 1960) This was shown experimentally by Jacobsen (1968) who did not find variations in the extinction of epithelial nuclei of the prostate when the thickness of the sections varied from 2 to 13 µm.

It is nevertheless of importance in nearly homogeneous nuclei that the cutting of the embedded tissue is as accurate as possible, so that the variations in thickness are within acceptable limits. The optical system should have so great a vertical resolving power that the depth of focus is smaller than the smallest variant in thickness of the tissue. This requires a great numeric aperture and a great total magnification (Einarson 1960) In addition, a sufficiently large number of measurements is necessary so that the thickness of the sections can be regarded as a statistical variable, the fluctuations of which are levelled out by the mean.

By measurements on the cytoplasm in gallocyanin-chromalum-stained tissue it has been shown (Pakkenberg 1958 Sandritter et al 1963 Jacobsen 1968) that Lambert's law is valid, i. e. that the extinction is proportional to the thickness of the section In studies of the concentration of RNA in the *cytoplasm* the thickness will then be a more significant factor

With the same adjustment of the microtome there is an appreciable variation in the thickness from section to section. Hallén (1962) found a variation of coefficient (standard deviation/mean × 100 %) of 28 % between different sections Pakkenberg (1958) found a variation of ± 50 % between different sections, and similar observations were reported by Holtet (1962)

Even in the same section there is some variation from area to area (Jacobsen 1968) even though it is considerably smaller than that between different sections. Hallén (1962) found a variation in measurements on the same section with a coefficient of variation of 6 % but his measurements were performed under optimal and far more accurate conditions than are normally used in the preparation of histological sections

The accuracy of the individual measurement by focusing is great. Brattgård (1954) found a coefficient of variation of 3 2 % while Lange & Engström (1954) found a coefficient of variation of about 10 % in thickness measurements on 5 µm sections, with a greater accuracy for thicker sections. Pakkenberg (1958) also reported that the focusing method yielded a great accuracy (± 0 06 µm) and similar observations were made by Hallén (1962): a coefficient of variation below 2 %

The variations between different sections is thus several times greater than those within the same section (Hallén 1962)

A large number of methods for the determination of the thickness of sections have been devised (Weissbach 1960) The earliest and most commonly used method is to focus on the upper and lower planes of the section recording the movements of the objective on a scale *(Hallén 1956) This method is* based on the investigations and calculations of focusing and vertical *resolving power performed by Abbé* and Berek (1927)

In measuring the thickness of a section it is of importance to use a great vertical power of resolution, which requires a great numeric aperture of the objective and a great total magnification (Berek 1927)

A mechanical method for measuring the thickness of the sections was elaborated by *Glimstedt & Håkansson (1951) who used a* microcator However this method involves a certain risk of mechanical deformation of the tissue even at the low pressure used in the measurements. The microcator was utilized for the registration of the small movements of the objective in determinations of the thickness of sections by Hallén (1956) Holtet (1962) and Jacobsen (1968)

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From each biopsy specimen, some sections were stained with haematoxylin-eosin for sur vey microscopy and with PAS. The thickness

of the sections was determined by the microcator technique used by Holtet (1962) and Jacobsen (1968) The thickness was measured in four different places in the bronchial epithelium, and sections of an average thickness between 6 and 8 μm were used for photometric measurements. As already mentioned, the thickness varies from place to place in the same section and particularly from section to section, and even the determination is subject to slight errors The determinations of the thickness were made to exclude major divergences The sledge microtome proved relatively stable, the thickness of the sections being in most cases within the range from 6 to 8 μm

Photometric Measurements

The photometric measurements were carried out by means of a Leitz cytophotometer (Einarson et al 1965) which is constructed on a principle described by Hansen & Einarson (1956) The photometric arrangement is based upon the Leitz Ortholux microscope fitted with a special condensor system The modification from normal microscopy consists in an attachment replacing the ordinary ocular section The measuring area is selected by a prism system placed in the image plane and arranged so that the light from the remaining field of view is relayed to a viewing eyepiece The image seen in this eyepiece shows the measuring field accurately outlined as a centrally placed black area. Measurements in the image plane may be performed by the use of an eyepiece micrometer The magnification is determined by the objective and the interchangeable image-forming lens of the photometric attachment.

The photo multiplier is connected to a variable stabilized power supply which also includes facilities for dark current compensation. The signal current from the multiplier tube (modified by dark current compensation) is measured on a light-spot galvanometer (Kipp & Zonen) The gradation on this instrument comprises two scales on which the percentage transmission and extinction may be read directly

The source of light is a low voltage microscope lamp, which is supplied from a stabilized power supply The light reaches the microscope through a Leitz monochromator which was checked at regular intervals by means of the spectral lines of a mercury lamp and proved to be very stable

The field of illumination may be selected by the field stop of the condensor system. The smallest field stop was used for all measurements. The lens system of the condensor was throughout the investigations a microscope objective × 25 with a numeric aperture of 0.50. The objective system of the microscope itself was in all measurements an oil immersion objective × 100 with a numeric aperture of 1.30

The interchangeable image forming lens of the photometric attachment was "Optics No. 3" for all the nuclear measurements, which in the sections corresponded to a measuring field of 2 5 μm in diameter while the illuminated area was 23 μm in diameter Leitz "Optics No 4" was used for the cytoplasmic measurements. This gives a measuring field of 1.5 μm in diameter

In nuclear measurements, the nucleic acid content may be calculated in arbitrary units (A U) as extinction multiplied by area. The cross sectional area of the spheric or ellipsoid nuclei was taken as $\pi d^2/4$ and $\pi d_1 d/4$ respectively The constant factor $\pi/4$ was omitted in all determinations The content of nucleic acids is thus stated as $d_1 d$ E, where d_1 and d designate the small and the large diameter and E the extinction

The diameters of the nuclei were as already mentioned, determined by a Leitz eyepiece micrometer for which the micrometer value with "Optics No 3" is 3 85 μm (i. e. 1 unit in the eyepiece micrometer corresponds to 3 85 μm) and with Optics No 4 2 35 μm

In small nuclei of normal epithelium and in certain tumours the extinction was measured in one place centrally in the nucleus. In larger tumour nuclei in which the distribution of the

chromatin may be more irregular the extinction is stated as the average of 3–5 determinations.

In the measurements of the thickness of sections by means of the microcator in connection with the Leitz Ortholux microscope, an oil-immersion object-objective × 100 with a numeric aperture of 1.30 and an eyepiece × 12, were used.

The nuclear measurements were performed at a wave length of 510 nanometres (nm) Both the gallocyanin-chromalum solution and gallocyanin-chromalum-stained nuclei have an absorption maximum at 570 nm. As the nuclei are of a homogeneous appearance with a very dark colour the extinction in the nuclei is high. In order to obtain extinction values within an appropriate range where the error of the photometer is relatively slight, the extinction of the nuclei was determined away from the absorption maximum at 570 nm. 510 nm was found to be appropriate as the extinction of the bronchial epithelial nuclei at this wave length is of a suitable magnitude.

The prerequisites for using bronchial biopsy specimens for photometric determinations were that a sufficient amount of tissue was available that the tissue was not contused in the area on which measurements were to be made and that it was possible to obtain sections of the tissue cut at right angles to the surface, or nearly so (Palkovits & Fischer 1968, page 167) Furthermore, it was required that desquamation of the superficial layer of the epithelium had not occurred, so that the morphology of the intact epithelium could be assessed The measurements on the normal bronchial mucosae were all made in the basal cell layer The nuclei are here more regular more homogeneous and more uniform in shape and size than in the more superficial nuclei. They are also smaller which results in fewer cut nuclei

Having ensured that there was no overlapping of the nuclei, and that the nucleus appeared intact in the section, the extinction was determined in the central plug, and the small and large diameters were read on the eyepiece micrometer No measurements were made on extremely elongated nuclei in which the large diameter was more than 1½ times the small diameter (Leuchtenberger 1958). The nucleic acid content was determined in 30 nuclei.

The cytoplasmic measurements were made at the absorption maximum of 570 nm. As the nuclei were often lying close to each other and the rim of cytoplasm was narrow the small measuring field with a diameter of 1.5 µm was used. The determinations in the normal mucosae were made on the cytoplasm in the basal cell layer which does not contain PAS-positive material. The basophilic substance was completely removed with RNase, which shows that it consists of cytoplasmic RNA. 50 measurements were made on each section with one or two determinations per basal cell nucleus. It is important to use careful focusing to ensure that there are no sectioned parts of nuclei in the area to be measured. In the metaplastic mucosae, 150 measurements were made on each section, viz. 50 measurements in the basal, 50 in the intermediate, and 50 in the superficial cell layer In the tumours, 150 cytoplasmic measurements were made, viz. 50 determinations in three different places in the tumour tissue.

Material

In a preceding investigation, the bronchial epithelium had been studied in human autopsy material. A characteristic feature was very extensive autolytic processes with desquamation of the superficial cell layers of the epithelium, so that only the basal cell layer was preserved. Accordingly it was not possible to assess the morphology of intact epithelium. Autopsy could not be made until 12 hours post mortem at the earliest. In an autopsy material examined 6 hours post mortem, Auerbach et al. (1956) found that denudation of the epithelium was a considerable disadvantage in the study of autopsy material, and that it prevented the

investigation in more than half the cases. Similar observations were made by Cunningham et al. (1959) The advantage of using biopsy material is thus that immediate fixation of small tissue fragments with preservation of the morphology becomes possible As compared with specimens removed at operation biopsy specimens offer the advantage that it is possible to obtain fragments of normal mucosae from patients who do not suffer from pulmonary or bronchial disease. The biopsy specimens of bronchogenic tumours were taken from the peripheral prominent part of the tumour i. e from parts in which relatively slight necrosis is present. As it was not possible to obtain sufficient fresh material of different bronchogenic tumours, old formalin fixed biopsy material from the Institute of Pathology was also used In all cases, the fresh biopsy material was fixed in formalin in order to obtain as uniform treatment of the tissue as possible.

All morphologically normal bronchial mucosae and about half of the metaplastic mucosae were fresh biopsy material. Of 75 biopsy specimens, 36 fulfilled the criteria for microphotometric determinations. The tumours were all obtained from the Institute of Pathology The adenomata and adenocarcinomata were from the period 1962–1968 while the squamous-cell carcinomata and the small celled anaplastic carcinomata and some of the metaplastic mucosae originated from the period 1962–1965 The material included 107 blocks, of which 57 could be used for microphotometric measurements. All sections were revised and assessed together with a pathologist.

Personal Investigations

The relative-quantitative photometric determinations of the nucleic acid content in the bronchial epithelium were carried out on gallocyanin-chromalum stained tissue The absorption curve for the gallocyanin-chromalum solution was determined in two layers of different thickness 0 3 mm and 1 0 mm The measurements were performed by means of a Zeiss spectrophotometer

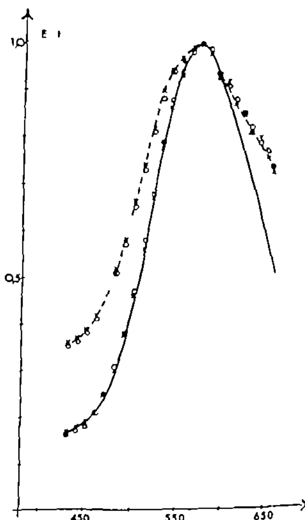


Fig. 1 —Absorption curves for gallocyanin-chromalum in solution (—O— and —×—) and tissue-bound (- - -O- - - and - - -×- - -)

The absorption curve for tissue bound gallocyanin-chromalum was determined on gallocyanin-chromalum-stained sections from two different bronchial specimens (Nos. 37 and 88) All values were related to each other by proportional calculation the absorption maximum fixed as the extinction value of 1 0 (fig. 1)

It is seen from the absorption curves for the gallocyanin-chromalum solution that the two curves for different thicknesses of the layers are identical which means that Lambert's law is valid for the gallocyanin-chromalum solution The absorption curves for the two nuclei are also identical (the two nuclei had a max

imal extinction of 0.90 and 0.94 respectively)

It is also seen that the tissue-bound gallocyanin-chromalum has an absorption curve occupying a higher level than that for the staining solution, especially in the short-wave area, but also, although to a lesser degree, in the long-wave area. The absorption maxima of gallocyanin-chromalum in solution and bound to nucleic acids are identical, i. e. there is no metachromasia.

The absorption curves for gallocyanin-chromalum in solution and in tissue have been determined by various investigators, who have reported similar results (Pakkenberg 1959 Sandritter et al. 1959) Both Sandritter et al. (1963) and Jacobsen (1968) found that the absorption curve for tissue-bound gallocyanin-chromalum in the short-wave area is somewhat higher than that for a gallocyanin-chromalum solution, while in the long-wave area it is only a little higher

Empirically it was found that the absorption in the nuclei of the bronchial epithelium at 510 nm, which was used for the nuclear measurements, was 73.5 % of that at the maximum of the extinction.

Errors of Measurements

Nuclear Measurements

The total nucleic acid content of the nuclei in the bronchial epithelium was calculated as the arithmetic mean of 30 non-enzyme-treated basal nuclei The values cluster around a mean with an average coefficient variation of 12.0 % (standard deviation/mean × 100). Figure 2 shows an example of the determination of the content of nucleic acids in the basal nuclei of a normal bronchial epithelium.

In order to check the accuracy measurements were performed on 30 nuclei in a normal bronchial epithelium. The measurements were repeated on the same section (No 28) at 4-hour intervals. The average content of nucleic acids was measured as 11.5 A. U 11.8 A. U 11.3 A. U and 11.3 A. U

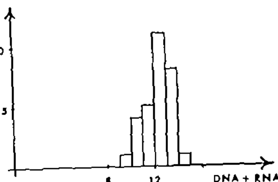


Fig. 2.—Nuclear nucleic acid content (section No. 22) Abscissa, nucleic acid content. Ordinate: number of nuclei. Mean 12.4 A.U standard deviation 1.1 A.U coefficient of variation 8.8 %

The maximal variation was 0.5 A. U., which in relation to the mean is a variation of 4.3 % i. e. good reproducibility exists within the individual section.

In a number of specimens the nucleic acid content in the nuclei was determined, both in different sections on the same slide and in sections mounted on different slides prepared at intervals of several months.

Table 1 *Nuclear nucleic acid content in different sections on the same slide*

Specimen No.	Section 1 A. U	Section 2 A. U	Variation A. U
28	11.5	11.5	0.0
37	12.4	12.6	0.2
41	14.3	14.0	0.3
44	13.6	14.3	0.7
55	11.2	12.7	1.5
57	14.1	13.2	0.9
61	13.0	13.4	0.4
62	12.9	12.6	0.3

In table 1 the average variation is 0.54 A. U which is an average variation of 4.1 % in relation to the mean.

From table 2 it appears that the average variation is 1.09 A. U., which is a variation of 8.7 % in relation to the mean.

In three cases, biopsy specimens of normal mucosae from different places of the bronchial tree were secured from the same patient (from

investigation in more than half the cases Similar observations were made by Cunningham et al (1959) The advantage of using biopsy material is thus that immediate fixation of small tissue fragments with preservation of the morphology becomes possible. As compared with specimens removed at operation, biopsy specimens offer the advantage that it is possible to obtain fragments of normal mucosae from patients who do not suffer from pulmonary or bronchial disease. The biopsy specimens of bronchogenic tumours were taken from the peripheral prominent part of the tumour i. e from parts in which relatively slight necrosis is present. As it was not possible to obtain sufficient fresh material of different bronchogenic tumours, old formalin-fixed biopsy material from the Institute of Pathology was also used In all cases, the fresh biopsy material was fixed in formalin in order to obtain as uniform treatment of the tissue as possible.

All morphologically normal bronchial mucosae and about half of the metaplastic mucosae were fresh biopsy material. Of 75 biopsy specimens, 36 fulfilled the criteria for microphotometric determinations. The tumours were all obtained from the Institute of Pathology The adenomata and adenocarcinomata were from the period 1962–1968 while the squamous-cell carcinomata and the small-celled anaplastic carcinomata and some of the metaplastic mucosae originated from the period 1962–1965 The material included 107 blocks, of which 57 could be used for microphotometric measurements. All sections were revised and assessed together with a pathologist.

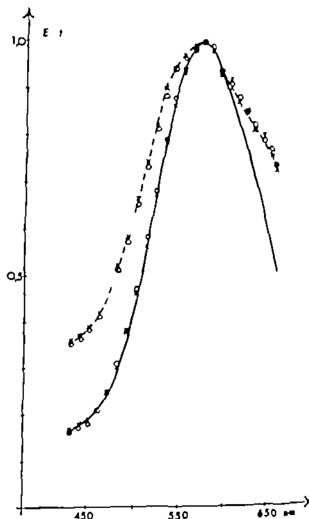


Fig. 1 —Absorption curves for gallocyanin-chromalum in solution (—O— and —×—) and tissue-bound (- - -O- - - and - - -×- - -)

Personal Investigations

The relative-quantitative photometric determinations of the nucleic acid content in the bronchial epithelium were carried out on gallocyanin-chromalum-stained tissue. The absorption curve for the gallocyanin-chromalum solution was determined in two layers of different thickness 0 3 mm and 1 0 mm The measurements were performed by means of a Zeiss spectrophotometer

The absorption curve for tissue-bound gallocyanin-chromalum was determined on gallocyanin-chromalum-stained sections from two different bronchial specimens (Nos. 37 and 88) All values were related to each other by proportional calculation, the absorption maximum fixed as the extinction value of 1 0 (fig 1)

It is seen from the absorption curves for the gallocyanin-chromalum solution that the two curves for different thicknesses of the layers are identical which means that Lambert's law is valid for the gallocyanin-chromalum solution The absorption curves for the two nuclei are also identical (the two nuclei had a max

urement on the individual section, which is probably due to variations in the thickness of the sections.

Table 5 *Cytoplasmic extinction in sections on different slides*

Specimen No	Section 1	Section 2	Variation
20	0.1827	0.1715	0.0112
22	0.1769	0.2006	0.0237
28	0.1603	0.1585	0.0018
31	0.1726	0.1739	0.0013
36	0.2046	0.1965	0.0081
59	0.1736	0.1485	0.0251

The average deviation is 0.0119 corresponding to a variation of the mean of 6.7 %. The average variation is of the same order as in table 4 and is probably due to variations in the thickness of the sections.

In three cases, biopsy specimens were secured from two different places of the bronchial tree in the same patient. The average values of the cytoplasmic extinction were as shown in table 6.

Table 6. *Average cytoplasmic extinction in specimens from two different places of the bronchial tree in the same patient*

Specimen No.	Section 1	Section 2	Variation
65–66	0.1777	0.1771	0.0006
67–68	0.1669	0.1552	0.0117
69–70	0.1[illegible]0	0.1461	0.0181

The average variation is 0.0101 corresponding to a variation in relation to the mean of 6.4 %.

While certain nuclear measurements in the fresh and older material can be directly compared (for example, the number of hyperdiploid and polyploid cell nuclei) because the values are relative, this cannot straight away be assumed as far as the cytoplasmic extinction measurements are concerned. In order to assess whether the deviating treatment of the older

Table 7 *Rabbit trachea. All specimens fixed in formalin water. Specimen No 4 fixed after 15 minutes in physiological saline. Specimens Nos 5 and 6 were prepared in the Institute of Pathology*

Specimen No.	Neutralized formalin	Formalin concentration (%)	Fixation (hours)	Rinsing (hours)	Toluol (hours)
1	–	2	24	24	2
2	+	2	24	24	2
3	+	4	24	24	2
4	+	4	24	24	2
5	+	2	2	0	0
6	+	2	24	0	0

material had had any measurable influence on the RNA concentration, the cytoplasmic concentration was determined in the tracheal epithelium of a rabbit which had been exposed to variations in the fixation and subsequent treatment (table 7). The pH in unneutralized formalin was determined as 4.3 (the low level being due to the formation of formic acid), while pH in freshly neutralized formalin was 7.6.

In sections 7 µm thick the cytoplasmic extinction was determined in 50 places in the cytoplasm around the basal cell nuclei. For each specimen, the extinction is stated as the average of determinations on one or two sections (table 8).

It is seen that the values of the cytoplasmic

Table 8 *Cytoplasmic extinction in rabbit trachea*

Specimen No.	Section 1	Section 2	Average
1	0.22		0.22
2	0.24		0.24
3	0.24	0.24	0.24
4	0.24		0.24
5	0.23		0.25
6	0.22	0.23	0.23
Average			0.24

Table 2 *Nuclear nucleic acid content in sections mounted on different slides*

Specimen No	Section 1 A. U	Section 2 A. U	Variation A. U
20	12.2	11 7	0.5
2.	12 4	13.2	0.8
28	11.5	9 6	1.9
33	12.6	11 6	1 0
36	11.5	12.2	0 7
41	14.3	13.2	1 1
59	13 4	15.0	1 6

the opening of the right middle lobe bronchus and that of the left lower lobe bronchus) The specimens from the same patient were sectioned and stained in the same procedure (table 3)

Table 3 *Nuclear nucleic acid content in different places of the bronchial tree in the same patient*

Specimen No	Section 1 A U	Section . A. U	Variation A U
65–66	11.2	12.6	1 4
67–68	10 9	12.8	1.9
69–70	13 4	13 1	0.3

The average variation is 1.2 A U corresponding to a variation in relation to the mean of 9 7 % i. e. of the same magnitude as the variations between sections on different slides of the same biopsy specimen

Cytoplasmic Measurements

The concentration of RNA in the cytoplasm of the basal cells was calculated as the arithmetic mean of 50 measurements for each section The values cluster around a mean with a coefficient of variation of about 30 % Thus, *the dispersion of the nucleic acid concentration* is considerably greater in the cytoplasm than in the nuclei

An example of *the determination of the* cytoplasmic concentration in a normal section is shown in figure 3

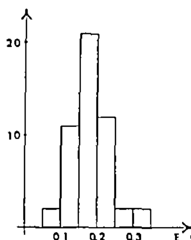


Fig. 3 —Cytoplasmic RNA concentration (section No 46). Abscissa, extinction. Ordinate: number of measurements. Mean 0.18, standard deviation 0 05 coefficient of variation 8 %

To check the accuracy of the measurements, the cytoplasmic concentration in the same epithelium was determined several times (specimen No 28)

The average cytoplasmic extinction was measured as 0 1603 0 1589 and 0 1563 i. e. the maximum variation was 0 0040 In relation to the mean, this is a variation of 2.5 % Thus good reproducibility exists as to measurements on cytoplasm

As in nuclear measurements, the cytoplasmic concentration was determined in a number of specimens, both in different sections on the same slide and on different slides (tables 4 and 5)

Table 4 *Cytoplasmic extinction in different sections on the same slide*

Specimen No	Section 1	Section 2	Variation
5	0.1603	0 1587	0 0016
73	0 1701	0 1653	0 0048
84	0 1771	0.1653	0 0118
87	0 1840	0 1601	0 0 39
88	0 1371	0 1492	0 01 1

The average variation is 0 0108 corresponding to a variation of the mean of 6 7 % The variation is larger than the errors of meas-

urement on the individual section, which is probably due to variations in the thickness of the sections.

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5	0.25		0.25
6	0.22	0.23	0.23
Average			0.24

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Specimen No	Section 1 A U	Section 2 A. U	Variation A. U
20	12.2	11 7	0.5
22	12.4	13.2	0.8
28	11.5	9 6	1 9
33	12.6	11 6	1 0
36	11.5	12.2	0 7
41	14.3	13.2	1 1
59	13 4	15 0	1 6

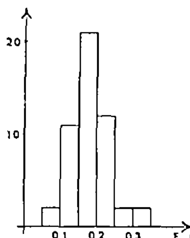


Fig. 3—Cytoplasmic RNA concentration (section No 46). Abscissa, extinction. Ordinate number of measurements. Mean 0 18, standard deviation 0 05 coefficient of variation 28 %

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The average variation is 0 0108 corresponding to a variation of the mean of 6 7 % The variation is larger than the errors of meas-

NORMAL BRONCHIAL EPITHELIUM

Histological Structure

The epithelial elements of the bronchial tree are endodermally developed from a ventral diverticulum on the foregut (Hamilton et al. 1962).

The respiratory epithelium of the major bronchi is a pseudo-stratified columnar epithelium with kinocilia. The cell nuclei are arranged in two or three layers, but all cells extend down to the basement membrane. The basal cells do not reach the surface. Goblet cells are scattered in the epithelium. The epithelium rests on a basement membrane. Below this membrane is the lamina propria with loose connective tissue, which contains numerous small glands of a mixed mucous-serous type. In the lamina propria are scattered lymphocytes and accumulations of lymphatic tissue, either diffuse or as nodules (Messerklinger 1958, Bloom & Fawcett 1962).

The connective tissue contains hyalin cartilage in the trachea and large bronchi in the form of C-shaped rings. The deepest layers consist of smooth muscle cells and dense connective tissue (Bloom & Fawcett 1962)

Histologically different types of cells have been described in the respiratory epithelium. Chang (1957) mentioned four different types of cells. basal, intermediate ciliated and goblet cells.

The nuclei of the basal cell layer are mainly spherical in shape (Messerklinger 1958) They are regular and compact, and the nucleolus does not form any distinct structure (Rhodin & Dalhamn 1956). The cytoplasm is sparse around the basal cells and contains many granula presumed to be RNA (Rhodin & Dalhamn 1956)

The ciliated cells and the goblet cells are highly differentiated (Messerklinger 1958) In addition to these types, and superficial to the basal cells, there are various types of cells differing in morphology with or without secretory granules and PAS-positive material in the cytoplasm (Rhodin & Dalhamn 1956, Blenkinsopp 1967 Bindreiter et al 1968)

DNA Content of Normal Cell Nuclei

If the DNA content is determined microphotometrically in a number of nuclei in a histological section, a certain variation in the DNA values around a mean will be found with a coefficient of variation ranging from 7 to 20 % (Swift 1953 Alfert & Swift 1953 Jobst & Sandritter 1961 Garcia 1962 a, Alpen & Johnston 1966)

It is difficult to decide whether this variation is due to a real difference in the DNA content or merely to measuring errors. Small variations in the DNA content can hardly be revealed on account of photometric measuring errors (Vendrely & Vendrely 1956, Walker & Richards 1959)

Beatty (1954) considered the existence of inconstancy likely Some somatic cells with an abnormal number of chromosomes may be at the end of their life span and do not divide any more, but most of the cells with an abnormal chromosome number will have divided several times. Swift (1966 b) revealed variations in the DNA content which, at least in many tissues, exceeded that which could be explained solely on the basis of photometric errors. The variations can be taken as an expression partly of measuring errors and partly as real variations because of an irregular number of chromosomes

concentration of RNA is about 0 24 The dif ference between the lowest and highest values (0.22 and 0 25) is not even slightly significant ($P > 0\ 10$ P referring to the probability of ex ceeding the stated limit of the null hypothesis)

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(1952 a) failed to disclose any content of RNA in normal cell nuclei, whereas chemical determination on the same nuclei revealed a nuclear RNA content of about 7.5 %

Cytoplasmic RNA

The concentration of cytoplasmic RNA may to a certain extent be assessed visually in a histological section when a stoichelometrically reacting dye is used. However microphotometric methods are more accurate, as the concentration of cytoplasmic RNA can then be measured.

As emphasized by Davies & Walker (1953) and Sandritter Pilny et al. (1966) the concentration of RNA in the cytoplasm can be measured. It is much more difficult to determine the total content of RNA in the cytoplasm of the cell usually it is extremely difficult, if not impossible, to assess the cytoplasmic volume in histological sections.

Quantitative photometric determinations of cytoplasmic RNA have been made, both in visible light on gallocyanin-chromalum-stained material and by ultraviolet absorption measurements on unstained material.

Determinations of cytoplasmic RNA on gallocyanin-chromalum-stained tissue have been carried out both on nerve cells (Einarson 1951 Krogh & Einarson 1954 Einarson & Krogh 1955 Hansen & Einarson 1956 Bech 1957 Pakkenberg 1958 1959 1960 1962 b, 1963 Falbe Hansen & Pakkenberg 1963 Thomsen & Pakkenberg 1962, Pakkenberg & Thomsen 1964 Pakkenberg & Vrå-Jensen 1964 Einarson et al. 1965), and on secreting cells (Oram 1955 Weber 1958 Holtet 196).

Ultraviolet absorption measurements have been performed on cytoplasm in various tissues, primarily by the Swedish school (Caspersson 1936, Hydén 1943 Thorell 1947) but also by many other investigators (Lagerstedt 1949 Sandritter 1953 Attardi 1957 Seed 1966 a, 1966 b, Sundelin 1967)

Only a few studies of the cytoplasmic RNA concentration in respiratory epithelium are on record. Bertalanffy (1964) visually evaluated the content of cytoplasmic RNA in the alveolar cells of the rat and found that it was increased in chronic inflammatory conditions.

Sandritter Müller et al. (1958) studied the cytoplasmic absorption of sputum cells by ultraviolet microphotometry The cytoplasmic content of RNA in the ciliated cells was very low as compared with that in the squamous cells from the oral mucosa. They did not report on the cytoplasmic RNA content of the basal cells of the bronchial epithelium.

In a preliminary report, the present author presented investigations of the RNA concentration in bronchial epithelial cells from morphologically normal and from metaplastic human bronchial mucosae (Greisen 1968).

Personal Investigations

Normal human bronchial epithelium consists of a ciliated pseudo-stratified columnar epithelium with the nuclei arranged in two or three layers. In the lamina propria, lymphocytes are nearly always present either as single, scattered cells or as small nodules. Sero-mucous glands are often found in the lamina propria in biopsy specimens.

Nuclear Measurements

A total of 36 biopsy specimens of morphologically normal bronchial epithelium were studied. In each specimen, the nucleic acid content was determined in 30 basal nuclei of bronchial epithelium which had not been treated with RNase and the average content of nuclear nucleic acid was then calculated. (*In the calculations a small amount of cytoplasmic RNA was included, see page 41*)

In normal bronchial epithelium, there are a few nuclei with a content of DNA which is increased as compared with the DNA content in the majority of the nuclei. These cells, which are synthetizing DNA in preparation for mitosis, were excluded in the calculations (see page 48). The fact that some cells are synthetizing DNA involves the risk of a positive

(Alfert & Swift 1953) In two-wave photometry Patau & Swift (1953) found a DNA content which was in complete agreement with the hypothesis of the constancy of DNA and regarded variations as an expression of photometric measuring errors. The same conclusion was reached by Pollister et al (1951)

In normal tissue, cells with an abnormal chromosome number will always be present (Koller 1958 Sandberg et al. 1960 Visfeldt 1966) They seldom deviate from the normal diploid cells by more than one chromosome (Hauschka 1961) Aneuploid cells are found only with a low frequency (Hsu 1961)

Ford (1960) found that 0 4 % of the cells in reticular tissue in mice had an abnormal number of chromosomes. Similar conditions are presumably present in human tissue (Ford 1960 Hamerton 1961)

In a study of normal bone marrow Sandberg et al (1961) observed as much as 12 % aneuploid cells produced by mitotic irregularities in the haemopoiesis. In normally regenerating liver tissue, Stich (1963) revealed 0–3 % cells with mitotic irregularities and 0–2 1 % cells with aneuploid DNA values.

DNA Content of the Bronchial Epithelial Nuclei

Determinations of the DNA content in normal bronchial epithelium have been made in connection with studies of the DNA content in bronchogenic tumours (Stowell 1946 Boriani & Gandolfi 1961 Sandritter Seidel et al 1965) These investigations show that the DNA content in the bronchial nuclei, like that of other cells, centres around a mean and that the average value in normal epithelium is considerably lower than that observed in bronchogenic tumours.

By means of ultraviolet microphotometry Sandritter Müller et al (1958) studied the nucleic acid content in various types of cell nuclei from scrapings of the bronchial mucosa and found that the average values for the basal and ciliated cells were equal.

In mice exposed to tobacco smoke, Leuchtenberger et al (1958) determined the average content of DNA in the bronchial epithelial nuclei in order to find out whether histochemical alterations could be detected before morphological changes in the epithelium occurred No such alterations in the average content of DNA were disclosed, but a slightly larger volume of the nuclei and an increased content of nuclear protein were found in the tobacco-exposed animals as compared with a control group

RNA Content of the Cell Nuclei

In the chemical determination of the RNA content in isolated nuclei it is difficult to avoid contamination with cytoplasmic RNA while, on the other hand, loss of RNA may occur in the isolation of the nucleus (Swift 1953)

According to the reports, the RNA content in different organs and in cells in different states of function varies widely The ratio between DNA and nuclear RNA is reported to be from 0 6 1 to 40 1 most values, however being within the range from 10 1 to 20 1 i e the nuclear RNA content is 5–10 % of the total nucleic acid content of the cell nucleus.

Microphotometric determinations of the nuclear RNA content can be made on tissue specifically stained for nucleic acids or by means of ultraviolet microphotometry The RNA content is then calculated as the difference between the amounts of nuclear nucleic acid without and with RNase treatment.

By ultraviolet microphotometry Sandritter Jobst & Rakow (1965) found that thymic lymphocytes had a nuclear RNA content corresponding to 7 8 % of the total nucleic acid content of the cell nucleus. In liver-cell nuclei Sandritter et al (1957) using both ultraviolet photometry and microphotometry on gallocyanin-chromalum stained nuclei found a nuclear RNA content of 10 % of the total nucleic acid content of the cell nucleus. By ultraviolet microphotometry Leuchtenberger et al

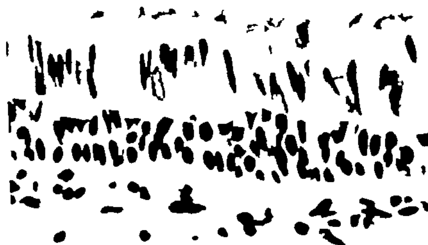


Fig. 6.—Normal bronchial epithelium (RNase, gallocyanin-chromalum, × 715)

Table 9. *Age and sex distribution of 33 patients with normal bronchial epithelium.*

Age years	Women	Men	Total
10–19	1	1	2
20–29	3	1	4
30–39	0	2	2
40–49	1	4	5
50–59	2	9	11
60–69	1	5	6
70–79		1	3
	10	[illegible]3	33

Table 10. *Nuclear nucleic acid content related to age. The range and average values are shown.*

Age years	No. of patients	Nuclear nucleic acid A.U	A erage A.U
10–19	2	10 9–12.8	11 9
20–29	4	11 1 14.2	12.9
30–39	2	11.2 11.5	11 4
40–49	5	10.7–14.6	12.4
50–59	11	10.2 14.1	12.8
60–69	6	11.2 14.1	12.4
70–79	3	12.1 14.4	12.9

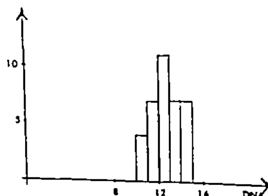


Fig. 7.—Distribution of the average nuclear nucleic acid content in morphologically normal bronchial epithelium.

Simple analysis of variance showed that $F < 1$ ($P > 0.50$) i.e. there is no significant differ ence between the groups. The variation between the age groups is smaller than that within the individual groups.

The total nuclear nucleic acid content is re lated to the diagnoses in the patients of the series of morphologically normal bronchial mucosae in table 11. Of the eight specimens of morphologically normal epithelium from patients with bronchogenic carcinoma, five were from patients with squamous-cell carcinoma, one from a patient with adenocarcinoma and two from patients with anaplastic carcinoma.

It is not possible to secure bronchial biopsy



Fig 4 —Normal bronchial epithelium (gallocyanin-chromalum, × 350).

skewness in the distribution curve of the basal cell nuclei. As the mitotic activity is low in bronchial epithelium this risk will be relatively slight when the nucleic acid content is calculated as the average of 30 randomly selected basal nuclei

The total nuclear nucleic acid content as the determination of the modal value of tumour-cell nuclei (Atkin et al 1966)

The average content of nuclear nucleic acid in the normal mucosae ranged from 10 2 to 14 6 A U., with an average of 12 6 A U for the entire series (fig 7)

The 36 morphologically normal bronchial mucosae originated from 33 patients, viz. 10 women and 23 men (table 9)

The total nuclear nucleic acid content as related to age is shown in table 10

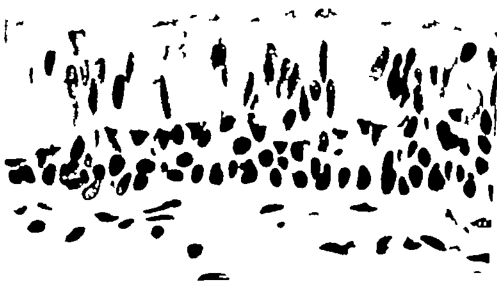


Fig. 5 —Normal bronchial epithelium (RNase gallocyanin chromalum, 715)

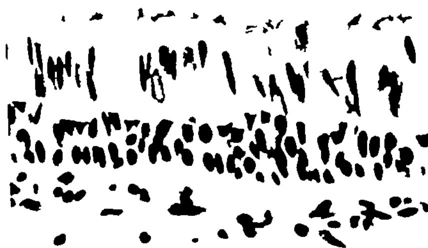


Fig. 6.—Normal bronchial epithelium (RNase gallocyanin-chromalum, × 715).

Table 9. *Age and sex distribution of 33 patients with normal bronchial epithelium.*

Age years	Women	Men	Total
10–19	1	1	2
20–29	3	1	4
30–39	0	2	2
40–49	1	4	5
50–59	2	9	11
60–69	1	5	6
70–79	2	1	3
	10	23	33

Table 10. *Nuclear nucleic acid content related to age. The range and average values are shown.*

Age years	No. of patients	Nuclear nucleic acid A. U	Average A. U
10–19	2	10.9–12.8	11.9
20–29	4	11.1–14.2	12.9
30–39	2	11.2–11.5	11.4
40–49	5	10.7–14.6	12.4
50–59	11	10.[illegible]–14.1	12.8
60–69	6	11.2–14.1	12.4
70–79	3	12.1–14.4	12.9

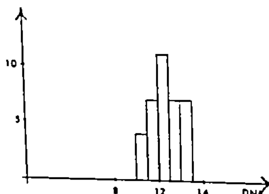


Fig. 7.—Distribution of the average nuclear nucleic acid content in morphologically normal bronchial epithelium.

Simple analysis of variance showed that $F < 1$ ($P > 0.50$), i.e. there is no significant difference between the groups. The variation between the age groups is smaller than that within the individual groups.

The total nuclear nucleic acid content is related to the diagnoses in the patients of the series of morphologically normal bronchial mucosae in table 11. Of the eight specimens of morphologically normal epithelium from patients with bronchogenic carcinoma, five were from patients with squamous-cell carcinoma, one from a patient with adenocarcinoma and two from patients with anaplastic carcinoma.

It is not possible to secure bronchial biopsy



Fig. 4.—Normal bronchial epithelium (gallocyanin-chromalum, × 350).

skewness in the distribution curve of the basal cell nuclei. As the mitotic activity is low in bronchial epithelium this risk will be relatively slight when the nucleic acid content is calculated as the average of 30 randomly selected basal nuclei.

The total nuclear nucleic acid content as the determination of the modal value of tumour-cell nuclei (Atkin et al 1966)

The average content of nuclear nucleic acid in the normal mucosae ranged from 10.2 to 14 6 A U with an average of 12 6 A U for the entire series (fig 7)

The 36 morphologically normal bronchial mucosae originated from 33 patients, viz. 10 women and 23 men (table 9)

The total nuclear nucleic acid content as related to age is shown in table 10

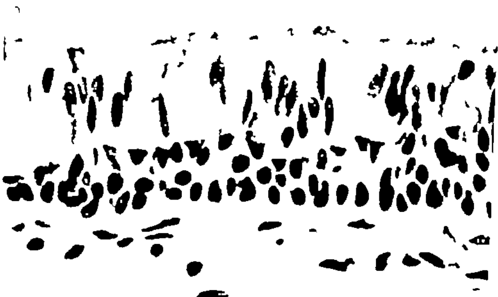


Fig. 5.—Normal bronchial epithelium (RNase gallocyanin chromalum, 715).

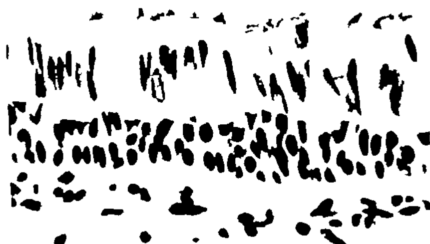


Fig. 6.—Normal bronchial epithelium (RNase, gallocyanin-chromalum, × 715).

Table 9 *Age and sex distribution of 33 patients with normal bronchial epithelium*

Age years	Women	Men	Total
10–19	1	1	2
20–29	3	1	4
30–39	0		
40–49	1	4	5
50–59	2	9	11
60–69	1	5	6
70–79		1	3
	10	23	33

Table 10 *Nuclear nucleic acid content related to age. The range and average values are shown.*

Age years	No. of patients	Nuclear nucleic acid A. U	Average A. U
10–19	2	10.9–12.8	11.9
20–29	4	11.1–14.2	12.9
30–39	2	11.2–11.5	11.4
40–49	5	10.7–14.6	12.4
50–59	11	10.2–14.1	12.8
60–69	6	11.2–14.1	12.4
70–79	3	12.1–14.4	12.9

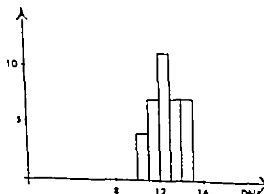


Fig. 7.—Distribution of the average nuclear nucleic acid content in morphologically normal bronchial epithelium.

Simple analysis of variance showed that F < 1 (P > 0.50), i.e. there is no significant difference between the groups. The variation between the age groups is smaller than that within the individual groups.

The total nuclear nucleic acid content is related to the diagnoses in the patients of the series of morphologically normal bronchial mucosae in table 11. Of the eight specimens of morphologically normal epithelium from patients with bronchogenic carcinoma, five were from patients with squamous-cell carcinoma, one from a patient with adenocarcinoma and two from patients with anaplastic carcinoma.

It is not possible to secure bronchial biopsy

Table 11 *Nuclear nucleic acid content in normal bronchial epithelium related to the diagnoses. The range and average values are shown*

Diagnosis	No. of patients	Nuclear nucleic acid A. U	Average A. U
Bronchogenic carcinoma	8	11.2–14.4	12.9
Pulmonary infiltration	5	11.2–14.6	12.6
Mediastinal cyst	3	10.7–14.0	12.5
Uveitis	6	11.5–14.2	12.8
Sarcoidosis (Boeck)	4	10.2–13.4	12.1
Chronic bronchitis	4	10.9–13.6	12.3
Atrophic rhinitis	1	10.9	10.9
Hodgkin's disease	1	13.8	13.8
Oesophageal carcinoma	1	12.2	12.2

specimens from perfectly healthy persons. However, the author succeeded in obtaining biopsy specimens from a group of patients with benign conditions unrelated to the bronchial system. These patients had no symptoms of bronchitis, bronchoscopy showed normal findings and the bronchial biopsy specimens secured consisted of morphologically normal bronchial mucosae. This group consisting of nine patients with mediastinal cysts or with uveitis without signs of sarcoidosis (Boeck) or other systemic disease served as controls.

Simple analysis of variance showed that $F < 1$ $(P > 0.50)$, i. e. there is no significant *difference between the groups. The variation* between the various groups is smaller than that within the individual groups.

Information was available of the smoking habits of eight of the nine subjects in the control group and of all the eight patients with bronchogenic carcinoma. In table 12, the use of tobacco is stated as + (less than 5 cigarettes daily), ++ (5–10 cigarettes daily), +++ (more than 20 cigarettes daily) and – (non-smokers).

It is seen that there is no difference in the amounts of the nuclear nucleic acid in the two groups.

In the cases where sufficient tissue was available, subsequent sections from each specimen were treated with RNase and the DNA content was measured on the basal cell nuclei. *In the control group and in the group of patients* with bronchogenic carcinoma and morphologically normal bronchial mucosae, it was *possible to measure the DNA content in seven* specimens from each group. Table 13 shows

Table 12 *Nuclear nucleic acid content in morphologically normal bronchial epithelium related to smoking habits. The range is shown in brackets*

	Control group		Patients with bronchogenic carcinoma	
Tobacco	No. of patients	Nuclear nucleic acid A. U	No. of patients	Nuclear nucleic acid A. U
–	6	12.9 (11.5–14.0)	0	
+	1	14.[illegible]	0	
++	1	10.7	5	13.0 (11.2–14.4)
+++	0		3	12.7 (11.5–14.1)
Average		1[illegible].8		1[illegible].9



Fig. 8.—Normal bronchial epithelium (gallocyanin-chromalum, 715)

Table 13. *Nuclear nucleic acid content in morphologically normal bronchial epithelium from patients with bronchogenic carcinoma and a control group*

	Average content of nuclear nucleic acid	
	RNA + DNA A.U	DNA A.U
Control group	12.7	12.0
Patients with bronchogenic carcinoma	12.9	11.8

the average total nuclear nucleic acid content in the two groups (nine subjects from the control group and eight patients from the group with bronchogenic carcinoma) and the DNA content in the same two groups (seven patients from each group).

From the table it appears that there is only a very slight difference in the amounts of nuclear nucleic acid in the two groups, and according to Student's t-test, the difference is far from being significant.

Cytoplasmic Measurements

The RNA concentration was measured around the basal nuclei in the 36 morphologically normal bronchial mucosae. For each section, the cytoplasmic extinction is stated as the average of 50 determinations.

The average extinctions in the sections ranged from 0.13 to 0.20, with an average of 0.17 for the entire series (fig. 10).

The average cytoplasmic extinction is related to age in table 14.

Simple analysis of variance showed that $F < 1$ $(P > 0.50)$ i.e. there is no significant difference between the groups. The variation

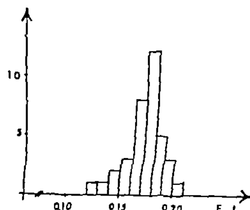


Fig. 10.—Distribution of the average cytoplasmic RNA concentration in morphologically normal bronchial epithelium.

Table 11 *Nuclear nucleic acid content in normal bronchial epithelium related to the diagnoses The range and average values are shown*

Diagnosis	No. of patients	Nuclear nucleic acid A U	Average A. U
Bronchogenic carcinoma	8	11.2–14 4	12.9
Pulmonary infiltration	5	11.2–14 6	12.6
Mediastinal cyst	3	10 7–14 0	12.5
Uveitis	6	11.5–14.2	12 8
Sarcoidosis (Boeck)	4	10.2–13 4	12.1
Chronic bronchitis	4	10.9–13 6	12.3
Atrophic rhinitis	1	10 9	10 9
Hodgkin s disease	1	13.8	13 8
Oesophageal carcinoma	1	12.[illegible]	1[illegible]2

specimens from perfectly healthy persons. However the author succeeded in obtaining biopsy specimens from a group of patients with benign conditions unrelated to the bronchial system These patients had no symptoms of bronchitis bronchoscopy showed normal findings and the bronchial biopsy specimens secured consisted of morphologically normal bronchial mucosae. This group consisting of nine patients with mediastinal cysts or with uveitis without signs of sarcoidosis (Boeck) or other systemic disease served as controls.

Simple analysis of variance showed that $F < 1$ ($P > 0\ 50$) i e there is no significant difference between the groups. The variation between the various groups is smaller than that within the individual groups

Information was available of the smoking habits of eight of the nine subjects in the control group and of all the eight patients with bronchogenic carcinoma. In table 12, the use of tobacco is stated as + (less than 5 cigarettes daily) ++ (5–10 cigarettes daily) +++ (more than 20 cigarettes daily) and − (non smokers)

It is seen that there is no difference in the amounts of the nuclear nucleic acid in the two groups.

In the cases where sufficient tissue was available, subsequent sections from each specimen were treated with RNase, and the DNA content was measured on the basal cell nuclei In the control group and in the group of patients with bronchogenic carcinoma and morphologically normal bronchial mucosae it was possible to measure the DNA content in seven specimens from each group Table 13 shows

Table 12 *Nuclear nucleic acid content in morphologically normal bronchial epithelium related to smoking habits The range is shown in brackets*

Tobacco	Control group: No of patients	Control group: Nuclear nucleic acid A. U	Patients with bronchogenic carcinoma: No of patients	Patients with bronchogenic carcinoma: Nuclear nucleic acid A. U
−	6	12.9 (11.5 − 14.0)	0	
+	1	14.2	0	
++	1	10 7	5	13 0 (11 − 14 4)
+++	0		3	12.7 (11.5 − 14 1)
Average		12.8		1[illegible].9

Table 16. *Cytoplasmic extinction in morphologically normal bronchial epithelium related to smoking habits. The range is shown in brackets*

Tobacco	Control group: No. of patients	Control group: Cytoplasmic extinction	Patients with bronchogenic carcinoma: No. of patients	Patients with bronchogenic carcinoma: Cytoplasmic extinction
–	6	0.18 (0.1603–0.1951)	0	
+	1	0.18 (0.1771)	0	
++	1	0.18 (0.1776)	5	0.18 (0.1435–0.2046)
+++	0		3	0.16 (0.1371–0.1802)
Average		0.18		0.17

the cytoplasmic RNA concentration in morphologically normal bronchial epithelium obtained from an uninvolved part of the bronchial tree in patients with bronchogenic carcinoma and from a control group.

RNase Treated Bronchial Epithelium

In the control group and in the group of patients with bronchogenic carcinoma, the nuclear nucleic acid content was measured in control sections treated with a phosphate buffer without RNase for 2 hours (pH 7.6, temperature 37 C).

Stowell & Zerzoli (1947) observed increased nuclear staining in buffer controls without RNase after fixation in Carnoy's solution, acetone and absolute alcohol. They presumed that the increased staining was due to adhesion to the nucleus of substances released from the cytoplasm. Toluidine blue and pyronine were used for the staining (pH not stated).

Jobst & Sandritter (1961) and Sandritter et al. (1963) found that treatment in a buffer for hours, with or without RNase gave rise to increased nuclear staining with gallocyanin-chromalum. They explained this phenomenon by release of phosphate groups now being available for the stain. Kaufmann et al. (1951) reported that fixed cells treated with RNase showed increased staining with acid dyes, but that the staining was eliminated by proteolytic enzymes, which proved that the increased staining was due to protein.

In a number of bronchial mucosae the nuclear nucleic acid content and the cytoplasmic RNA concentration were determined in the ordinary gallocyanin-chromalum-stained sections and in the above mentioned control sections (tables 17 and 18).

These studies show that as far as the bronchial epithelium is concerned, no alterations occur in the nucleic acid content in the nuclei or in the cytoplasm when the tissue is treated in a phosphate buffer for 2 hours (pH 7.6, temperature 37 C). The differences observed are very slight and far from being significant.

Table 17. *Nuclear nucleic acid content in morphologically normal non-enzyme treated bronchial epithelium.*

Specimen No.	Nuclear nucleic acid A. U	Nuclear nucleic acid in control sections A. U	Difference A. U
22	12.4	14.0	+1.6
26	10.7	12.8	+2.1
28	11.5	11.8	+0.3
33	1[illegible].6	12.3	−0.3
36	11.5	11.5	0.0
50	14.4	13.4	−1.0
52	12.1	11.7	−0.4
54	14.0	12.9	−1.1
55	11.2	11.[illegible]	0.0
57	14.1	12.1	−[illegible].0
59	13.4	13.6	+0.2
84	14.[illegible]	13.2	−1.0
87	1[illegible].9	13.9	+1.0
88	11.5	14.2	+[illegible].7
Total			+2.1
Average difference			+0.15

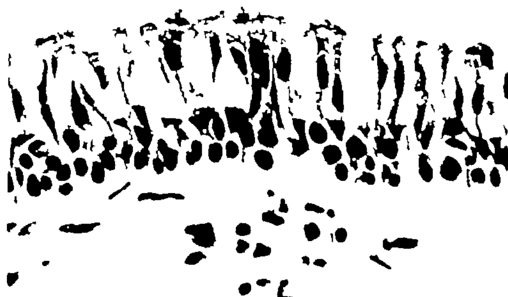


Fig. 9 —Normal bronchial epithelium (gallocyanin-chromalum. × 715).

between the age groups is smaller than that within the individual groups

As in the nuclear measurements, the cytoplasmic extinction is related to the diagnoses of the patients (table 15)

Simple analysis of variance showed that F < 1 (P > 0 50) i e there is no significant difference between the groups.

The cytoplasmic extinction is related to the use of tobacco in the control group and in the group of patients with bronchogenic carcinoma in table 16

It is seen that there is only a very slight difference between the groups, and that it is the reverse of what might have been expected The difference is far from being significant.

Table 14 *Average cytoplasmic extinction related to age The range is shown in brackets*

Age years	No of patients	Cytoplasmic extinction
10–19	2	0 16 (0 1610–0 1681)
20– 9	4	0.17 (0.1 80–0 198)
30–39	2	0 17 (0 1603–0 1777)
40–49	5	0.17 (0.1589–0 1776)
50–59	11	0 18 (0 1435–0.2046)
60–69	6	0 17 (0.1371–0 18 6)
70–79	3	0.18 (0.1696–0 18?7)

Conclusions

The nuclear nucleic acid content and the cytoplasmic RNA concentration in morphologically normal bronchial mucosae are distributed evenly around a mean of 12 6 A U and 0 17 respectively No evidence was revealed in favour of the assumption that the total nucleic acid content in the cell nuclei or the cytoplasmic RNA concentration should vary with the age diagnosis or tobacco consumption of the patients. No difference was demonstrated between the nuclear nucleic acid content and

Table 15 *Average cytoplasmic extinction related to the diagnoses The range is shown in brackets*

Diagnosis	No of patient	Cytoplasmic extinction
Bronchogenic carcinoma	8	0 17 (0 1371–0 046)
Pulmonary infiltration	5	0 17 (0 1550–0 1878)
Mediastinal cyst	1	0 18 (0 1776–0 1840)
Uveitis	6	0 18 (0.1603–0 1951)
Sarcoidosis (Boeck)	4	0 16 (0 1 80–0 198)
Chronic bronchitis	4	0 17 (0.155 –0 174)
Atrophic rhinitis	1	0 17 (0 1681)
Hodgkin disease	1	0 19 (0 1934)
Oesophageal carcinoma	1	0 18 (0 1827)

Table 16. *Cytoplasmic extinction in morphologically normal bronchial epithelium related to smoking habits The range is shown in brackets*

	Control group		Patients with bronchogenic carcinoma	
Tobacco	No. of patients	Cytoplasmic extinction	No. of patients	Cytoplasmic extinction
−	6	0.18 (0 1603–0 1951)	0	
+	1	0 18 (0.1771)	0	
+ +	1	0.18 (0 1776)	5	0 18 (0 1435–0.2046)
+ + +	0		3	0 16 (0.1371–0 1802)
Average		0.18		0 17

the cytoplasmic RNA concentration in morphologically normal bronchial epithelium obtained from an uninvolved part of the bronchial tree in patients with bronchogenic carcinoma and from a control group

RNase-Treated Bronchial Epithelium

In the control group and in the group of patients with bronchogenic carcinoma, the nuclear nucleic acid content was measured in control sections treated with a phosphate buffer without RNase for 2 hours (pH 7 6 temperature 37 C)

Stowell & Zorzoli (1947) observed increased nuclear staining in buffer controls without RNase after fixation in Carnoy's solution, acetone and absolute alcohol. They presumed that the increased staining was due to adhesion to the nucleus of substances released from the cytoplasm. Toluidine blue and pyronine were used for the staining (pH not stated)

Jobst & Sandritter (1961) and Sandritter et al (1963) found that treatment in a buffer for 2 hours, with or without RNase, gave rise to increased nuclear staining with gallocyanin-chromalum They explained this phenomenon by release of phosphate groups now being available for the stain. Kaufmann et al. (1951) reported that fixed cells treated with RNase showed increased staining with acid dyes, but that the staining was eliminated by proteolytic enzymes, which proved that the increased staining was due to protein.

In a number of bronchial mucosae the nuclear nucleic acid content and the cytoplasmic RNA concentration were determined in the ordinary gallocyanin-chromalum-stained sections and in the above mentioned control sections (tables 17 and 18)

These studies show that as far as the bronchial epithelium is concerned, no alterations occur in the nucleic acid content in the nuclei or in the cytoplasm when the tissue is treated in a phosphate buffer for 2 hours (pH 7 6, temperature 37 C) The differences observed are very slight and far from being significant.

Table 17 *Nuclear nucleic acid content in morphologically normal non-enzyme-treated bronchial epithelium*

Specimen No	Nuclear nucleic acid A. U	Nuclear nucleic acid in control sections A. U	Difference A. U.
22	12.4	14 0	+1.6
26	10 7	12 8	+2.1
28	11.5	11.8	+0.3
33	12.6	12.3	−0.3
36	11.5	11.5	0.0
50	14.4	13 4	−1.0
5?	12.1	11 7	−0 4
54	14 0	12.9	−1 1
55	11.2	11.2	0.0
57	14.1	12.1	−2.0
59	13.4	13.6	+0.2
84	14	13.2	−1.0
87	12.9	13.9	+1.0
88	11.5	14.2	+2.7
Total			+2.1
Average difference			+0.15

The cytoplasmic extinction was also measured in a number of sections around the basal nuclei, both without and with RNase treatment (table 19)

It is seen that the RNA content in the cytoplasm disappears completely after RNase treatment. This shows that the stained substance is RNA, and that gallocyanin-chrom alum specifically stains cytoplasmic RNA in the basal cells in the normal bronchial epithelium

Nuclear RNA Content

By measuring the total nuclear content of nucleic acid in the bronchial epithelium and the DNA content in the RNase treated sections, the RNA content of the nucleus may be calculated.

The studies were carried out on morphologically normal bronchial mucosae from the control group and the group of patients with bronchogenic carcinoma. For each patient, the content of nucleic acid is stated as the average of the total nucleic acid content in sections prepared in the ordinary way and in the control sections (tables 20 and 21)

Table 18 *Cytoplasmic extinction in non-enzyme-treated morphologically normal and metaplastic sections Metaplastic sections are marked m*

Specimen No.	Cytoplasmic extinction	Cytoplasmic extinction in control sections	Difference
22	0 18	0 18	0.00
26	0 18	0.17	−0 01
28	0 16	0 18	+0.02
33	0 17	0 17	0.00
50	0 18	0.15	−0.03
52	0 17	0 16	−0 01
54	0 14	0 15	+0.01
55	0 17	0 16	−0 01
57	0 18	0 18	0 00
88	0 14	0 15	+0 01
37 m	0.29	0.29	0 00
41 m	0.29	0.27	−0.02
44 m	0.21	0.23	+0.02
55 m	0.22	0.22	0 00
Total			−0 02
Average difference			−0 0014

Table 20 *Nuclear nucleic acid content in the control group*

Specimen No.	DNA + RNA A. U	DNA A. U	RNA A. U
22	13.20	12.8	0.40
26	11 75	11.9	−0 15
8	11 65	11.0	0.65
52	11.90	10.6	1.30
59	13.50	12.0	1.50
84	13 70	12.1	1 60
87	13 40	13 7	−0.30
Total	89 10	84 1	5 00

Table 19 *Cytoplasmic extinction in the basal cells in morphologically normal bronchial epithelium and in the subepithelial intercellular connective tissue Without and with RNase treatment*

Specimen No.	No RNase treatment		After RNase treatment	
	Basal cells	Connective tissue	Basal cells	Connective tissue
22	0.18	0 03	0 04	0 03
26	0.18	0.03	0 04	0 03
28	0.16	0 04	0 03	0 02
37	0.16	0.03	0.05	0 03
44	0 16	0.02	0 03	0 03
55	0.17	0.03	0.03	0.03
56	0.17	0 04	0 03	0 03
57	0 18	0.04	0 05	0 05
59	0.17	0.03	0 03	0 03
61	0 17	0.03	0 0[illegible]	0 03
87	0.18	0.0[illegible]	0 04	0.03

Table 21 *Nuclear nucleic acid content in the patient group with bronchogenic carcinoma.*

Specimen No.	DNA + RNA A.U	DNA A.U	RNA A.U
33	12.45	10.8	1.65
34	11.50	11.4	0.10
50	13.90	13.2	0.70
54	13.45	12.0	1.45
55	11.20	11.2	0.00
57	13.10	11.8	1.30
88	12.85	12.5	0.35
Total	88.45	82.9	5.55

The average nuclear RNA content was 0.71 A.U corresponding to 5.6 % RNA in the control group, and 0.79 A.U in the group with bronchogenic carcinoma corresponding to a nuclear RNA content of 6.3 %.

In these calculations, however, is included a factor conditional on the extinction in the cytoplasm surrounding the nucleus. The thickness of the nuclei in the bronchial epithelium (average 4.6 µm, see later) is not as great as that of the section (7 µm). On the assumption that the rim of cytoplasm is perfectly homogeneous, the cytoplasmic share of the extinction can be eliminated (Swift & Rasch 1956). If the basal nuclei are rotary ellipsoids (Abercrombie 1946, Grundmann et al. 1961 a) which sections cut parallel to the epithelial surface also suggest, the height of the cytoplasmic rim will be equal to the thickness of the section minus the short diameter of the nucleus. The average short and long diameters and the average extinction were calculated for each section. The cytoplasmic extinction and the unspecific cytoplasmic extinction after RNase treatment were measured. As the cytoplasmic extinction was measured at 570 nm, and the nuclear extinction at 510 nm, the cytoplasmic extinction must be corrected by the factor 0.735 (see page 27). Finally, the weak unspecific absorption of light in the over- and underlying rim of cytoplasm must be subtracted from the total nuclear extinction.

If E_t is the total extinction, E_k = the nuclear extinction, E_c = the cytoplasmic extinction, E_c = the corrected cytoplasmic extinction, d = the thickness of the section and a = the short diameter of the nucleus, then the following relation will be valid.

$$E_t = E_k + \frac{d - a}{d} \times E_c$$

Table [illegible] *Corrected cytoplasmic extinction in the control group*

Specimen No	Average: Short diameter	Average: Extinction	Unspecific absorption	Corrected extinction	E_c (%)
2	1.[illegible]4	0.687		0.678	
Cytoplasm	0.58	0.18	0.04	0.033	4.9
6	1.76	0.640		0.631	
Cytoplasm	0.36	0.18	0.04	0.032	5.1
8	1.[illegible]4	0.568		0.559	
Cytoplasm	0.58	0.16	0.04	0.028	5.0
52	1.05	0.819		0.807	
Cytoplasm	0.77	0.17	0.04	0.041	5.1
59	1.33	0.618		0.612	
Cytoplasm	0.49	0.17	0.03	0.028	4.6
84	1.14	0.758		0.747	
Cytoplasm	0.68	0.18	0.04	0.038	5.1
87	1.09	0.825		0.813	
Cytoplasm	0.73	0.18	0.04	0.041	5.0
Average					5.0

Table 23 *Corrected cytoplasmic extinction in the patient group with bronchogenic carcinoma*

Specimen No.	Average Short diameter	Average Extinction	Unspecific absorption	Corrected extinction	E_r (%)
33	1.26	0.553		0.544	
Cytoplasm	0.56	0 17	0 04	0 030	5.5
36	1 15	0 620		0 609	
Cytoplasm	0 67	0.20	0 04	0 043	7 1
50	1 20	0 686		0 676	
Cytoplasm	0 62	0 18	0 04	0 035	5.2
54	1.27	0.579		0.570	
Cytoplasm	0.55	0 14	0 04	0 022	3 9
55	1 14	0 652		0 644	
Cytoplasm	0 68	0 17	0 03	0 038	5 9
57	1 15	0.622		0 608	
Cytoplasm	0 67	0.18	0 05	0.035	5 8
88	1.22	0.706		0 696	
Cytoplasm	0 60	0 14	0 04	0 024	3 4
Average					5.3

which means that E_r constitutes

$$\frac{\frac{d-a}{d} \times E_r}{E_t} \times 100\,\%$$

of the total extinction (Swift & Rasch 1956)

That part of the total nuclear RNA content which is constituted by cytoplasmic RNA can now be calculated (tables 22 and 23)

It appears that the cytoplasmic RNA con centrations in the two patient groups constitute 5 0 % and 5 3 % of the total uncorrected nu clear RNA content. This was calculated to be 5 6 % and 6 3 % respectively of the total nu clear nucleic acid content.

If the cytoplasmic rim is perfectly homogeneous, the nuclear RNA content as a per centage of the total nucleic acid content of the nucleus will thus constitute.

5 6 % 5 0 % = 0 6 % in the control group and
6 3 % − 5 3 % = 1 0 % in the patient group with bronchogenic carcinoma.

The figures are too small to allow a statis tical comparison of the content in the two groups.

In the explanation of the low nuclear RNA content the following possibilities must be considered.

1 That actually only a small amount of RNA is present in the basal nuclei of the normal bronchial epithelium
2 That some nuclear RNA has been washed out of the nuclei during the preparation of the tissue.

It is likely that a certain loss of nuclear RNA occurs during the preparation of histological sections, primarily of s-RNA which constitutes about one third of the nuclear RNA content (Amano 1967 a 1967 b) (see page 14)

3 That some nuclear RNA is fixed so firmly by the formalin that it cannot be removed by RNase treatment

This is likely (see page 18)

4 That the average thickness of the section is not 7 μm

As mentioned on page 22 measurements of the thickness of the sections are subject to some uncertainty and in practice it is not possible to check the thickness of the sections in all areas where the cytoplasmic

extinction is measured. However the thickness of the sections was within the limits of 6–8 μm, and the average for all sections was very close to 7 μm.

5. That the RNA content is reduced in the control sections as compared with the sections prepared in the ordinary way which would result in a lower total nuclear extinction.

   This was not the case (see table 19)

6. That the staining capacity should be increased in the nuclei after treatment in phosphate buffer with and without RNase.

   This was not the case (see table 18)

7. That the calculated cytoplasmic share of the total extinction is too high. This is most likely. The calculated correction is only accurate if the cytoplasm is perfectly homogeneous, so that the extinction is proportional to the thickness of the cytoplasmic rim. The RNA in the cytoplasm is not perfectly homogeneous, but particulate, and that part of the cytoplasmic rim which lies above and below the nucleus will not participate in the total extinction to the same great extent as that part of the section which is inside the depth of focus (Einarson 1960) (see page 22)

   It is not possible to assess the magnitude of the error introduced into the calculations by this phenomenon.

The conclusion of the photometric determinations of the RNA content in the basal cell nuclei of the morphologically normal bronchial epithelium is that it is probably within the range from 1 % to 6 % of the total nucleic acid content in the nucleus, but the calculations are subject to some uncertainty

Dimensions of the Basal Nuclei

In the two groups of morphologically normal bronchial mucosae the average size of the measured nuclei was calculated (table 24)

Table 24 *Average size of the basal nuclei in the morphologically normal bronchial epithelium.*

	Diameter		
	Short μm	Long μm	Area μm²
Control group	4.58	6.16	22.2
Patients with bronchogenic carcinoma	4.62	6.47	23.5

The nuclei in the basal cell layer were not selected with a view to determining their size. Nuclei of irregular shape and excessively elongated nuclei were excluded. It is seen that the nuclei in the two groups differed only slightly in size, and the difference is not significant. It appears that the long diameter was 1.34 and 1.40 times as great as the short diameter in the two groups.

The area of the measuring field in the nuclear measurements was 4.9 μm² (see page 24), i.e., on an average it represented one quarter of the area of the nucleus. The measuring field was the largest possible which could be used for the nuclear measurements, as regard had to be paid to the short diameters of the smallest nuclei.

Table 23 *Corrected cytoplasmic extinction in the patient group with bronchogenic carcinoma*

Specimen No.	Average Short diameter	Average Extinction	Unspecific absorption	Corrected extinction	E_r (%)
33	1.26	0.553		0.544	
Cytoplasm	0.56	0.17	0.04	0.030	5.5
36	1.15	0.620		0.609	
Cytoplasm	0.67	0.20	0.04	0.043	7.1
50	1.20	0.686		0.676	
Cytoplasm	0.62	0.18	0.04	0.035	5.2
54	1.27	0.579		0.570	
Cytoplasm	0.55	0.14	0.04	0.022	3.9
55	1.14	0.652		0.644	
Cytoplasm	0.68	0.17	0.03	0.038	5.9
57	1.15	0.622		0.608	
Cytoplasm	0.67	0.18	0.05	0.035	5.8
88	1.[illegible]2	0.706		0.696	
Cytoplasm	0.60	0.14	0.04	0.0[illegible]4	3.4
Average					5.3

which means that E_c constitutes

$$\frac{\frac{d-a}{d} \times E_c}{E_t} \times 100\ \%$$

of the total extinction (Swift & Rasch 1956)

That part of the total nuclear RNA content which is constituted by cytoplasmic RNA can now be calculated (tables 22 and 23)

It appears that the cytoplasmic RNA concentrations in the two patient groups constitute 5.0 % and 5.3 % of the total uncorrected nuclear RNA content. This was calculated to be 5.6 % and 6.3 % respectively of the total nuclear nucleic acid content.

If the cytoplasmic rim is perfectly homogeneous, the nuclear RNA content as a percentage of the total nucleic acid content of the nucleus will thus constitute:

5.6 % − 5.0 % = 0.6 % in the control group and
6.3 % − 5.3 % = 1.0 % in the patient group with bronchogenic carcinoma.

The figures are too small to allow a statistical comparison of the content in the two groups.

In the explanation of the low nuclear RNA content the following possibilities must be considered.

1 That actually only a small amount of RNA is present in the basal nuclei of the normal bronchial epithelium

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It is likely that a certain loss of nuclear RNA occurs during the preparation of histological sections, primarily of s-RNA which constitutes about one third of the nuclear RNA content (Amano 1967 a, 1967 b) (see page 14)

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This is likely (see page 18)

4 That the average thickness of the section is not 7 µm

As mentioned on page 22, measurements of the thickness of the sections are subject to some uncertainty and in practice it is not possible to check the thickness of the sections in all areas where the cytoplasmic

1966) It seems reasonable to assume that its duration in man does not differ from that of other mammals (Lushbaugh 1956).

The mitotic index indicates the number of cells in mitosis at a given time. A high mitotic index may mean an increased number of mitoses or a prolonged mitotic time (Mazia 1961)

In adult tissue, the mitotic index will be an expression of the number of cells desquamated when no growth of the tissue occurs (Bertalanffy & Lau 196? a) Possibly the cell renewal is a protective mechanism. Many tissues are exposed to influences of various kinds, mechanical, enzymatic and bacterial. This is, however not the only cause of the cell renewal, as mitotic activity is present in various tissues which are unaffected by exogenous factors (Bertalanffy 1960) The cell renewal is a characteristic property of the tissue concerned rather than an expression of exogenous influences (Johnson 1961). After the cessation of growth, many tissues are still able to proliferate, while in others the cells have little or no ability to divide (Thrasher 1966)

The death of the host organism will prevent new cells from entering mitosis, while cells which have entered mitosis will continue this without delay (Lushbaugh 1956, Bertalanffy & Lau 196_ a) After death a steep fall in the number of mitoses occurs, and immediate fixation of the tissue is therefore of the greatest importance (Bertalanffy & Lau 1962 a). Only surgically removed tissue and biopsy specimens fixed immediately after removal are suitable for the determination of the mitotic activity (Bertalanffy 196).

The mitotic activity will show some diurnal fluctuations (Messier & Leblond 1960 Koburg & Maurer 196_) especially in slowly renewing tissues, while rapidly renewing cell systems will show only minor variations in the duration of the cell cycle and in the duration of the various phases (Thrasher 1966) Those parts of the digestive tract which are covered with columnar epithelium have no appreciable diurnal fluctuation, whereas parts covered with stratified squamous epithelium show some fluctuation, so that the mitotic activity is least after an active period and greatest after sleep (Bertalanffy 1960)

The mitotic activity may be influenced by various locally irritant or toxic substances (Bertalanffy & Lau 1962a) Prolonged fasting may also affect the mitotic activity (Hunt 1957 Hooper & Blair 1958, Bertalanffy & Lau 1962 a)

Methods of Estimation of Mitotic Activity

The mitotic activity can be estimated in different ways:

1 Counting of mitoses in fixed and stained tissue.
2. Counting of mitoses after colchicine treatment.
3 Autoradiography after treatment with radioactive thymidine (thymidine-H³).
4 Counting of cells with an increased DNA content.

Direct counting of mitoses in tissues with selection of cells in mitosis as assessed by purely morphological criteria may be beset with considerable difficulty and great uncertainty

In most tissues, the number of dividing cells is not sufficient for direct counting of mitoses to give significant data regarding cell division (Bertalanffy & Lau 1963)

Direct counting of mitoses in histological sections requires good fixation and staining. Counting of mitoses may be very difficult in tissues with small dark nuclei (Leblond et al. 1959) Mitoses can hardly be recognized in some tissues, and it may also be difficult to detect mitoses by microscopic examination (Messier & Leblond 1960, Schultze & Oehlert 1960). In neuroglia, for example, mitoses are very rarely seen. Studies with radioactive thymidine have, however shown that cell division does occur It is possible that mitoses cannot be seen on account of the small size of the cell, or the division may be atypical and im-

MITOSES

Most cells divide by mitosis, and the division comprises the cell nucleus and cytoplasm

During prophase, increasing coiling and condensation of the chromosomes take place. The nucleolus disappears, and duplication of the chromosomes into two chromatids occurs (Mazia 1961 DeRobertis et al. 1965) In early prophase, the chromosomes are evenly distributed later in prophase they approach the nuclear membrane. The mitotic spindle is formed in this phase or in metaphase. At the end of prophase the nuclear membrane disappears. At metaphase the chromosomes are disposed in the equatorial plane connected to the fibres of the mitotic spindle The chromatids move away from each other towards the poles, at the beginning of anaphase At telophase, this migration comes to an end the reconstruction of the nucleus begins the chromosomes become less compact, and the nuclear membrane is re formed. At the same time the cytoplasm is dividing.

The preparation for mitosis takes place in interphase a doubling of the DNA content occurs before visible signs of mitosis are present (Swift 1953 Patau & Swift 1953 Deeley et al 1954 Vendrely & Vendrely 1956 Deeley et al 1957 Mazia 1961)

The interphase is divided into three periods.

1 the G_1 phase or presynthetic gap
2 the S phase, in which DNA synthesis takes place and
3 the G phase or postsynthetic pause (Alpen & Johnston 1967)

During mitosis, no DNA is synthetized. The doubling of the DNA content occurs roughly along a straight line, i. e at a constant rate of synthesis (Swift 1953 Deeley et al 1957 Mazia 1961 Koburg & Maurer 1962 Maceira et al 1966) According to Alpen & Jo nston (1967), the doubling of DNA occurs along a slightly S-shaped curve, as the rate of synthesis is lower both at the beginning and at the end of the S phase. Mittermayer et al. (1968 a, 1968 b) found that the DNA synthesis was a little slower at the beginning than later in the S phase.

The time for DNA synthesis is usually stated to be of the order of 7½ hours (Thrasher 1966) with a range of variation from 6 to 9 hours (Deeley et al. 1957 Koburg & Maurer 1962 Yang et al. 1966 Maceira et al 1966, Remery et al 1967 Blenkinsopp 1968) occasionally it lasts a little longer (Lipkin et al. 1963 Stryckmans et al 1966 Blenkinsopp 1968, Mittermayer et al 1968 b)

The duration of mitosis and the rate of DNA synthesis seem to be relatively constant for a given cell type, whereas interphase and especially the G_1 phase may vary (Knowlton et al. 1950 Lushbaugh 1956 Mazia 1961 Meyer zum Gottesberge & Koburg 1963 Maceira et al 1966)

Mitosis is preceded not only by DNA synthesis, but also by a postsynthetic gap, the G_2 phase, which in man is usually shorter than the G_1 phase (Prescott 1961) A small percentage of the cells may have a deviating form of cell division in which the G_2 phase may be very long, possibly lasting for several days In mouse epidermis, 5 to 10 % of the basal cells show this form of cell division (Gelfant 1966)

The duration of mitosis in different tissues is nearly always from ½ to 3 hours (Mazia 1961) most frequently from ½ to 1 hour (Lushbaugh 1956 Bertalanffy 1964 Thrasher

Allen (1962) determined the mitotic index in most cells in the rat by counting mitoses and the cells which by *visual* evaluation had a DNA content that was twice normal. The DNA content had beforehand been measured microphotometrically in a certain number of cells. Most of them had a certain DNA content, and some cells the double content. A few cells contained an amount of DNA between these values.

Quenser et al. (1968) used microphotometric measurements of the DNA content for the determination of the mitotic index in human lymph nodes. Among a small number of cells, 104 68 and 58 respectively those with an increased DNA content outside the distribution curve for normal diploid cells were counted.

However also within the normal range of distribution for diploid cells, there will be some cells which are synthetizing DNA (Sod-Moriah et al. 1968).

By ultraviolet microphotometry Sandritter Muller et al (1958) examined scrapings of bronchial epithelial cells. The limit of normal nucleic acid content was fixed half way be t een the diploid and the tetraploid value, and the number of cells with an increased content of total nucleic acid was related to this limit. Of normal basal cells, 4 % and of ciliated cells, 14 % had a DNA content above this limit (stated for totals of 76 and 35 cells, respectively) The number of cells with an increased content of nucleic acid is remarkably high and must be taken with some reservation on account of the small number of cells studied In particular the percentage of ciliated cells seems large as DNA synthesis and mitosis should not occur in such cells.

Microphotometric measurements of the DNA content in RNase-treated, gallocyanin-chromalum-stained tissue have been used for the determination of the mitotic activity in human bronchial epithelium (Gresen 1968)

By microphotometric measurements, it is possible to find the cells with an increased DNA content a compared with normal diploid are synthetizing DNA, or in which DNA synthesis has come to an end, can be detected. This applies to cells in the S and G_2 phases of the interphase, and to cells at prophase and metaphase.

Mitotic Activity of Respiratory Epithelium

Cells which are responsible for the renewal of many tissues are chiefly of the less differentiated type, basal cells, reserve cells or germinative cells (Bertalanffy 1963).

The renewal of cells of the bronchial epithelium occurs from the basal cells. Mitoses are rarely or never seen in the highly differentiated cells (Messerklinger 1958).

Most ciliated cells are produced by mitosis in the basal cells, and this probably also applies to goblet cells. Mitoses in the differentiated cells (ciliated and goblet cells) are extremely rare, if they do occur at all (Bertalanffy 1963) Using H^3-thymidine Bindreiter et al. (1968) found that only the basal cells could accomplish division, whereas the goblet cells and ciliated cells were unable to divide. Blenkinsopp (1967) reported that when a basal cell divided, it formed one basal and one superficial cell, while a superficial cell at the same time divided into two superficial cells.

The cell renewal in the duodenum and oesophagus in mice probably occurs in such a manner that one stem cell divides, forming two stem cells, and at the same time one stem cell differentiates into a specialized cell (Thrasher 1966).

In the respiratory tract epithelium, the daily mitotic rate (the percentage of cells dividing in 24 hours) as determined by the colchicine method in the rat trachea is 2.1 % and in the bronchi 3 7 % corresponding to a total renewal of the cell population in 47 6 and 26.7 days, respectively (Bertalanffy & Lau 1962 a)

In the alveolar cells of the adult rat, Bertalanffy (1964) found a daily mitotic rate of 13.8 % and, by direct counting of mitoses in the alveolar cells, a mitotic activity of 0 48 %. For the alveolar cells, this corresponds to a duration of mitosis of about 50 minutes.

possible to recognize (Messier & Leblond 1960) The same applies to mammary epithelium (Sod Moriah et al 1968)

It may be attended with great difficulty to distinguish cells at prophase and telophase, as there is no definite morphological boundary towards the G_2 and the G_1 phase of interphase Of the cells in *mitosis, 70–80 % will be at* prophase or telophase, and on account of this it is understandable that direct counting of mitoses at least in many tissues, is beset with considerable uncertainty (Bertalanffy 1964)

In the epithelium of the small intestine, the mitotic activity is very brisk and in this field a number of investigations utilizing direct counting of the mitoses have been performed (Leblond & Stevens 1948 Bertalanffy & Nagy 1961 Padykula et al 1961)

On exposure to the action of colchicine or related substances, mitosis is arrested at meta phase By counting the number of metaphases in a tissue, its mitotic activity can be assessed. Colchicine inhibits the formation of the mitotic spindle. The chromatids are then unable to move away from each other In addition, substances inhibiting the spindle formation induce a contraction of the chromosomes, which renders them even more distinct under the mi croscope (*Östergren 1944 Ludford 1953 Hsu* 1961)

If the dose of colchicine is too large this will result in cell destruction inhibition of the *mitotic activity (Lushbaugh 1956)* and inhibi tion of DNA synthesis (Gustafsson 1968)

On *the other hand,* a too small dose of colchicine will prevent division of only some of the cells, and the inhibition of mitosis will be only transient (Lushbaugh 1956 Bertalanffy & Lau 1962 a) If a suitable dose is admin istered neither stimulation nor depression of the mitotic activity nor affection of DNA syn thesis, will occur (Bloch 1953 Bertalanffy & Leblond 1953 Hooper 1961 Gelfant 1963 Bertalanffy 1964)

By mitotic counts after colchicine treatment, the number of cells dividing in 24 hours, and hence the rate of renewal of *the tissue may* be calculated (Bertalanffy 1963)

Colcemide which is less toxic and more effective than colchicine, has been used for the determination of the mitotic activity in man (Laavåg 1967)

Thymidine H^3 is most commonly used for autoradiographic determinations of the mitotic ac tivity Cells specifically incorporate thymidine during the synthesis of DNA which is then stable (Amano et al 1959 Thrasher 1966)

Radioactive thymidine exerts a biological effect on dividing cells, and at the same time expose them to irradiation. Thymidine H^3 may increase the duration of metaphase, suppress DNA synthesis and delay cell division (Thrasher 1966 Yang et al. 1966 Fakan 1969)

The method is mainly of importance in ani mal experiments and in tissue cultures. In man the method can usually not be applied in vivo because of irradiation hazards (Oliver et al. 1960 Johnson et al 1961) In a few patients with incurable cancers, the mitotic activity has, however been determined after administration of labelled thymidine (Johnson et al 1960 Cole & *McKalen 1961 Lipkin et al. 1963)*

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Cells which at a given moment have an in creased DNA content as compared with a nor mal diploid cell population can be distin guished by photometric measurements.

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The cell renewal in the duodenum and oesophagus in mice probably occurs in such a manner that one stem cell divides, forming two stem cells, and at the same time one stem cell differentiates into a specialized cell *(Thrasher 1966)*

In the respiratory tract epithelium, the daily mitotic rate (the percentage of cells dividing in 24 hours) as determined by the colchicine method in the rat trachea is 2.1 % and in the bronchi 3.7 % corresponding to a total renewal of the cell population in 47.6 and 26.7 days, respectively (Bertalanffy & Leu 1962 a)

In the alveolar cells of the adult rat, Bertalanffy *(1964)* found a daily mitotic rate of 13.8 % and, by direct counting of mitoses in the alveolar cells, a mitotic activity of 0.48 %. For the alveolar cells, this corresponds to a duration *of mitosis* of about 50 minutes.

possible to recognize (Messier & Leblond 1960) The same applies to mammary epithelium (Sod Moriah et al 1968)

It may be attended with great difficulty to distinguish cells at prophase and telophase as there is no definite morphological boundary towards the G_2 and the G_1 phase of interphase Of the cells in mitosis, 70–80 % will be at prophase or telophase, and on account of this it is understandable that direct counting of mitoses at least in many tissues, is beset with considerable uncertainty (Bertalanffy 1964)

In the epithelium of the small intestine the mitotic activity is very brisk and in this field a number of investigations utilizing direct counting of the mitoses have been performed (Leblond & Stevens 1948 Bertalanffy & Nagy 1961 Padykula et al. 1961)

On exposure to the action of colchicine or related substances, mitosis is arrested at metaphase. By counting the number of metaphases in a tissue, its mitotic activity can be assessed. Colchicine inhibits the formation of the mitotic spindle The chromatids are then unable to move away from each other In addition substances inhibiting the spindle formation induce a contraction of the chromosomes, which renders them even more distinct under the microscope (Östergren 1944 Ludford 1953 Hsu 1961)

If the dose of colchicine is too large, this will result in cell destruction, inhibition of the mitotic activity (Lushbaugh 1956) and inhibition of DNA synthesis (Gustafsson 1968)

On the other hand, a too small dose of colchicine will prevent division of only some of the cells, and the inhibition of mitosis will be only transient (Lushbaugh 1956 Bertalanffy & Lau 1962 a) If a suitable dose is administered, neither stimulation nor depression of the mitotic activity nor affection of DNA synthesis, will occur (Bloch 1953 Bertalanffy & Leblond 1953 Hooper 1961 Gelfant 1963 Bertalanffy 1964)

By mitotic counts after colchicine treatment, the number of cells dividing in 24 hours, and hence the rate of renewal of the tissue, may be calculated (Bertalanffy 1963)

Colcemide, which is less toxic and more effective than colchicine, has been used for the determination of the mitotic activity in man (Liavåg 1967)

Thymidine H^3 is most commonly used for autoradiographic determinations of the mitotic activity Cells specifically incorporate thymidine during the synthesis of DNA which is then stable (Amano et al 1959 Thrasher 1966)

Radioactive thymidine exerts a biological effect on dividing cells, and at the same time expose them to irradiation Thymidine-H^3 may increase the duration of metaphase, suppress DNA synthesis and delay cell division (Thrasher 1966 Yang et al. 1966 Fakan 1969)

The method is mainly of importance in animal experiments and in tissue cultures. In man the method can usually not be applied in vivo because of irradiation hazards (Oliver et al 1960 Johnson et al 1961) In a few patients with incurable cancers the mitotic activity has, however been determined after administration of labelled thymidine (Johnson et al. 1960 Cole & McKalen 1961 Lipkin et al. 1963)

After in-vitro incorporation of H^3-thymidine, the mitotic activity can be determined in biopsy specimens (Fabrikant & Cherry 1969) Some reluctance must, however be recommended in the evaluation of DNA synthesis in removed tissue (cf page 45) (Bertalanffy & Lau 1962 a, Bertalanffy 1962)

By the thymidine-H^3 method the cells which incorporate radioactive thymidine during the synthesis of DNA in the S phase are revealed

Cells which at a given moment have an increased DNA content as compared with a normal diploid cell population can be distinguished by photometric measurements.

Cells with DNA values in the range of twice the normal diploid value are presumed to be cells synthetizing DNA in preparation for mitosis (Queisser et al 1966)

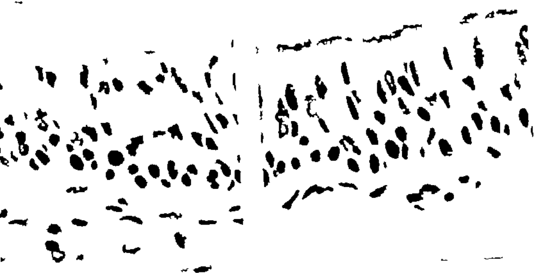


Fig. 11

Fig. 12.

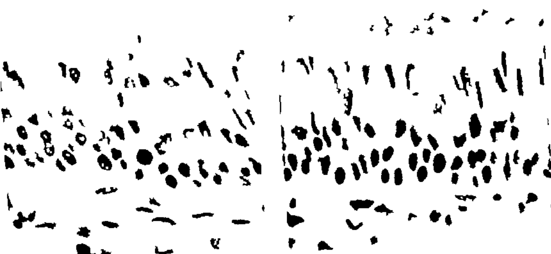


Fig. 13.

Fig. 14

Hyperdiploid cell nuclei in normal bronchial epithelium (RNase gallocyanin-chromalum, × 715).

doubled DNA content in preparation for mitosis would be distributed around the double of the diploid mean with the same scatter (Fig. 15).

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On the assumption that the duration of mitosis in the trachea and bronchi is the same as in the alveolar cells, it can be calculated from these figures that the number of mitoses at a given moment in the trachea and bronchi will be 0 07 and 0 13 % respectively ($2\ 1 \times 0\ 48$ 13 8 and $3\ 7 \times 0\ 48$ 13 8) If it is assumed that the duration of synthesis is about 7 times as long as that of mitosis (Thrasher 1966) 0 49 and 0 91 % of the cells will be in the S phase

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H^3-thymidine will be available for the synthesis of DNA for 3/4 to 1 hour (Koburg 1960 Mazia 1961) The labelled cells will be an expression of the cells incorporating H^3-thymidine in DNA during that period i.e cells in the S phase plus cells which have entered the G_2 phase within 3/4 to 1 hour

Personal Investigations

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In the study of the DNA content of the nuclei in the basal cell layer of the bronchial epithelium a few nuclei with an increased content of DNA were observed. These nuclei were darker and larger than the normal basal nuclei (figs. 11 12 13 and 14)

About one half of the cells with increased DNA content were found in the deepest part in the basal cell layer close to the basement membrane, while the other half were situated just superficial to the most basal cell layer On the other hand, no cells with increased DNA content were found in the more differentiated cells in the superficial cell layer

By counting the cells with increased DNA content, the number of cells which are synthetizing DNA or which have doubled the DNA content in preparation for mitosis, can be assessed.

By the determination of the DNA content in 20 to 30 nuclei after exclusion of those with a distinctly elevated DNA content, there will be only a slight risk of a positive skewness of the distribution curve of the DNA content in the diploid cells, because the mitotic activity in the bronchial epithelium is comparatively low (Atkin et al. 1966)

The DNA content will be distributed around a mean with only a little scatter (average coefficient of variation 8 7 %) If this was due exclusively to errors of measurements, cells with

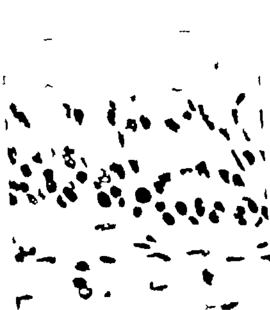


Fig. 11



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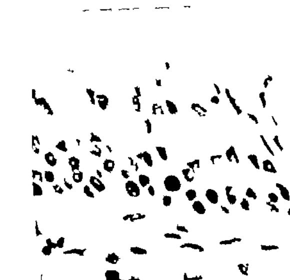


Fig. 13

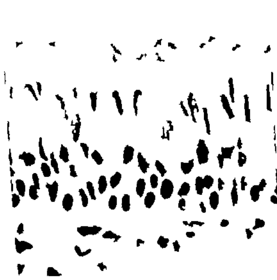


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Hyperdiploid cell nuclei in normal bronchial epithelium (RNase gallocyanin-chromalum, × 715).

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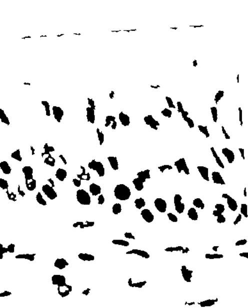
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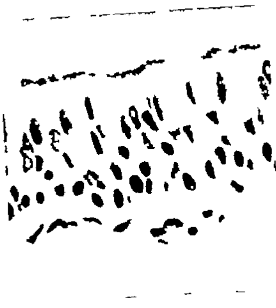
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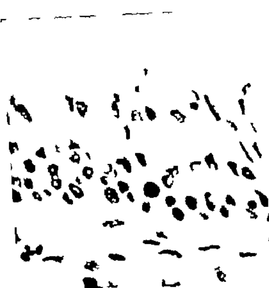
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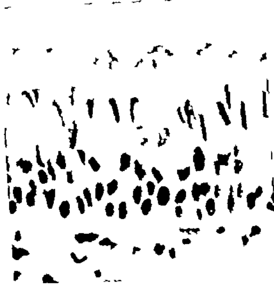
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The DNA content will be distributed around a mean with only a little scatter (average coefficient of variation 8.7 %) If this was due exclusively to errors of measurements, cells with

Table 25 *Age distribution average DNA content and number of hyperdiploid cells in the control group*

Section No.	Age years	DNA A.U	No. of cells counted	Hyperdiploid cells No.	Hyperdiploid cells per 1000
22	59	12.8	1107	5	4.5
26	45	11.9	2000	6	3.0
28	37	11.0	2000	6	3.0
52	71	10.6	1796	4	2.2
59	46	12.0	2000	6	3.0
84	28	12.1	2000	7	3.5
87	24	13.7	2000	5	2.5
U 3	75	10.2	2000	6	3.0
U 8	50	8.3	2000	5	2.5
U 15	68	11.7	2000	3	1.5
U 18	54	10.6	2000	7	3.5
U 19	50	9.5	2000	6	3.0
Average	50.6				2.9

does not fluctuate, that the synthetic rate is constant, and that the cells divide at the very moment they have doubled their DNA content, such a cell population synthetizing DNA will show a rectangular distribution (fig. 18). The zero is placed half-way between the low and the high level.

If in measurements on a cell selected at random, which has a value between $-a$ and $+a$, fortuitous variations (including measuring errors) in the normal distribution must also be accepted, it can be shown that the composite distribution law in which the individual value is designated x, can be written in a simple form. Further simplification is obtained by putting the standard deviation σ equal to 1 (which is very close to that found in the material).

The probability density function is determined by the formula.

$$g(x, a) = \frac{1}{2a}\,\frac{1}{\sqrt{2\pi}}\int e^{-u^2/2}\,du = \frac{1}{2a}\left[F(x+a) - F(x-a)\right]$$

where F(x) represents values of the prescribed

Table 26 *Age distribution average DNA content and number of hyperdiploid cells in the group of patients with bronchogenic carcinoma*

Section No	Age years	DNA A.U	No. of cells counted	Hyperdiploid cells No.	Hyperdiploid cells per 1000
33	58	10.8	2000	5	2.5
34	51	11.4	2000	7	3.5
50	72	13.2	2000	7	3.5
54	52	12.0	2000	1	0.5
55	69	11.2	2000	6	3.0
57	60	11.8	2000	7	3.5
88	67	12.5	2000	7	3.5
S 1	53	10.2	2000	5	2.5
S 9	55	12.0	2000	9	4.5
S 19	54	11.2	2000	6	3.0
S 20	54	1 8	2000	6	3.0
Average	58.5				3.0

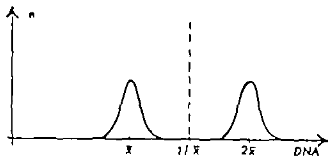


Fig. 15.—Distribution of DNA content in diploid and tetraploid nuclei in normal bronchial epithelium

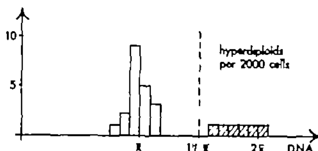


Fig. 16.—Distribution of DNA content in normal and hyperdiploid nuclei in normal bronchial epithelium (section No 59).

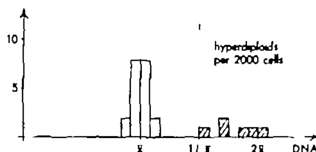


Fig. 17.—Distribution of DNA content in normal and hyperdiploid nuclei in normal bronchial epithelium (section No 55).

DNA above the content in the normal diploid cell nuclei were measured. All cells which in subsequent calculations had a DNA content of 1½ × the mean or more were stated as hyperdiploid cells (figs. 16 and 17).

The number of hyperdiploid cells is stated per 2,000 superficial and basal cells in the bronchial epithelium. In a few cases, however, they are stated for a figure less than 2,000, viz. when an insufficient number of nuclei were present in the section.

The mitotic activity was determined in the following groups:

1. Patients with normal bronchial mucosa and benign disease unrelated to the bronchial tree (control group).
2. Morphologically normal bronchial mucosa from patients with bronchogenic carcinoma.

In the original biopsy material the average age of the patients in the control group was 44.3 years as compared with 61.3 years in the second group.

To obtain more comparable groups as regards age and to examine a larger number of specimens, the original material was supplemented by biopsy specimens obtained from the Institute of Pathology. Group 1 was supplemented by specimens from patients with uveitis, group 2 by specimens from patients with bronchogenic carcinoma. An attempt was made to select elderly patients for group 1 and younger for group 2.

Tables 25 and 26 show the age distribution, average DNA content and number of hyperdiploid cells in the two groups.

It appears that there is only a slight difference in the average ages of the two groups, and that there is no difference in the average mitotic activity in normal bronchial epithelium in the control group and the group with bronchogenic carcinoma elsewhere in the bronchial mucosa.

A homogeneous cell population with a certain DNA value will, in preparation for mitosis, synthetize DNA until twice the original value is reached. Provided that the mitotic activity

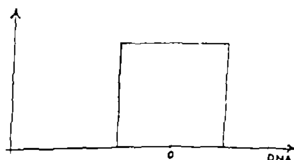


Fig. 18.—DNA content in synthetizing cells (see text).

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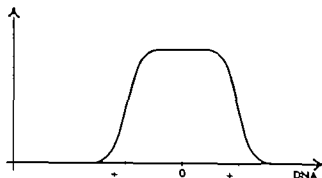


Fig. 19 —DNA content in synthetizing cells (see text)

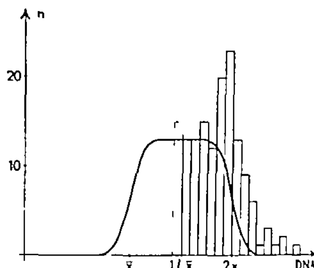


Fig. 20 —Distribution of hyperdiploid cell nuclei in morphologically normal bronchial epithelium

normal distribution function Figure 19 shows graphically the appearance of g (x n) In the figure, n = 6 which roughly corresponds to an average diploid DNA content of 12 in the normal nuclei, and a standard deviation of 1

After DNA synthesis there is however a postsynthetic pause, during which the cells have a tetraploid content of DNA This is manifested by an accumulation of cells with the tetraploid DNA content in the distribution curve.

The DNA content of the hyperdiploid cell nuclei in different sections cannot be directly compared As appears from tables 25 and 26 the diploid mean differs in the various sections. If the DNA content of the hyperdiploid cell nuclei is calculated in relation to a diploid mean of 12 A U the DNA content in the cells can be compared.

In the morphologically normal bronchial mucosae a total of 132 hyperdiploid cell nuclei were found These were distributed as shown in figure 20

If the hyperdiploid cell nuclei in the metaplastic mucosae are included the number increases to 257 (fig. 21)

It appears from figures 20 and 21 that, after a certain level there will be an accumulation of hyperdiploid cells around the tetraploid

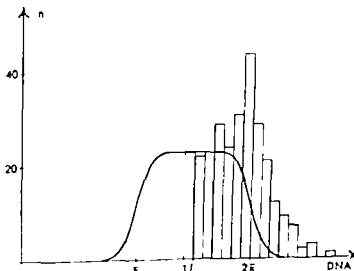


Fig 21 —Distribution of hyperdiploid cell nuclei in normal and metaplastic bronchial epithelium

value. If the cells divided immediately after the cessation of synthesis, the curve would assume a form as shown in figure 19 It can be seen from the curve that, of the 132 hyper diploid cells in the normal mucosae, 77 are inside the area that would be expected if the cells divided immediately whereas the accumulation of cells with the tetraploid value comprises 55 cell nuclei. As the curve in figure 19 is symmetrical around the mid-line, 77 cell nuclei must also be located to the left of the value $1^1/_2 \times$ the mean, corresponding to about 60 % (77 132 $\times$ 100)

When the normal bronchi contained a number of hyperdiploid cell nuclei corresponding to 0.3 % of the total number the total of cells which at a given moment are, or have been, synthetizing DNA will be 60 % higher i.e. about 0.48 % This number is of the same order as that stated for the mitotic activity in the respiratory epithelium in animals.

Conclusions

By means of microphotometry it has proved possible, in a simple way to investigate the mitotic activity in a tissue.

The method is harmless as no pre-treatment of the tissue is required. There is no need for planning the investigation beforehand, and older blocks of biopsy specimens can be used.

The investigation was carried out on human bronchial epithelium, in which the mitotic activity has not previously been studied.

The investigation revealed that there was no difference in the mitotic activity in the normal bronchial epithelium from a control group and a group of patients with bronchogenic carcinoma elsewhere in the bronchial mucosa. This means that disturbances in the mitotic activity in morphologically normal bronchial mucosae cannot be demonstrated prior to the development of precancerous changes.

METAPLASTIC BRONCHIAL EPITHELIUM

By metaplasia is understood the tranformation from one well-defined type of epithelium to another In the bronchial epithelium, meta plasia indicates the presence of transitional or stratified squamous epithelium. Basal-cell hy perplasia and atypical epithelial changes are, however usually also included in the meta plastic changes of the bronchial mucosa (Na sell 1963)

Metaplasia is caused by various factors, such as infections or damage of a chemical or phy sical nature During the development of meta plasia, normal epithelium passes through vari- ous phases The epithelium first shows an in- crease in the number of goblet cells (Spencer 1962) The formation of transitional epithelium may occur through a phase of splitting of the epithelium with formation of a slit between the basal-cell layer and the overlying col umnar-cell layer which shows different de- grees of degenerative changes, or through a phase of basal-cell hyperplasia (Nasiell 1963) Basal-cell hyperplasia may be defined as a condition with more than two layers of basal cells (Auerbach et al. 1956)

Through these phases, true metaplastic epi thelium develops, either transitional epithelium which is the most common or stratified squa mous epithelium Morphologically transitional epithelium is similar to the urinary tract epi- thelium. It is without cilia on the surface, and consists of several layers of polygonal cells, possibly with a few layers of flat cells on the surface. In stratified squamous epithelium there is a more pronounced flattening of the cells The transition from one type to the other occurs gradually (Nasiell 1963)

Through a stage of atypical epithelium car cinoma *in situ develops*. The atypical epithe lium is hypercellular and consists of polymor phic cells with hyperchromatic nuclei and an irregular chromatin pattern. In carcinoma in situ, the cellular atypia is more pronounced and occurs in all layers of the epithelium. The cells are carcinoma-like, but show no signs of invasive growth. Atypical epithelial changes and carcinoma in situ are almost exclusively found in metaplastic epithelium (Nasiell 1968 a, 1968 c)

It is presumed that the malignant neoplastic condition has a long preceding stage with mor phological changes in the cells of the epithe- lium and that carcinoma represents the final stage. These antecedent epithelial changes may be diffuse, while the invasive carcinoma in- volves only a limited area. The precancerous changes in the bronchial epithelium are cellular atypia and carcinoma in situ preceded by metaplasia. However the extent to which metaplasia and precancerous cell atypia de velop in parallel or to which metaplasia pre- cedes the atypical changes in the cells is un- known It is generally believed that metaplasia occurs first, and metaplasia without atypical cells is therefore not regarded as a pre- cancerous alteration (Nasiell 1968 c)

Most authors assume that metaplastic changes play an important part in the develop- ment of bronchogenic carcinoma and the view has repeatedly been expressed that there is a relation between metaplastic changes and lung cancer (Auerbach et al 1956 Auerbach et al. 1957 a, Valentine 1957 Nasiell 1968 a) in such a manner that bronchogenic carcinoma is preceded by these changes.

Metaplasia possibly promotes carcinogenesis In areas without cilia, the transport of secre tion is slow and they will therefore be partic

ularly exposed to an intensified action of carcinogens (Hilding 1956)

If the factors producing metaplasia are not combined with the carcinogenic factors which produce atypical cells, there is no risk of the development of carcinoma. The risk of malignant alterations increases with the extent of the metaplastic changes, because epithelium with metaplastic changes is more susceptible to carcinogenic influences than normal respiratory epithelium. Definitely atypical epithelium is virtually never encountered in normal respiratory pseudo-stratified columnar epithelium. Most frequently the atypical changes are seen as localized alterations within a wide spread area with metaplastic epithelium (Nasiell 1968 b).

Metaplastic changes in the bronchial epithelium, the preceding degenerative alterations in the columnar epithelium and atypical metaplasia occurred much more frequently in a series of squamous-cell carcinomata and undifferentiated carcinomata than in a control group and a group with adenocarcinomata (Hackensellner 1957 b, Nasiell 1963) The high incidence of metaplasia in squamous-cell carcinoma and undifferentiated carcinoma indicates that exogenous factors are responsible for the development of metaplasia, and that these alterations in the bronchial epithelium presumably constitute the basis for the development of squamous-cell carcinoma and undifferentiated carcinoma (Nasiell 1968 a) These findings are also in agreement with the assumption that adenocarcinoma of the bronchi to a lesser degree than squamous-cell carcinoma and undifferentiated carcinoma is associated with exogenous factors (Nasiell 1963 1966, 1967 Maamies 1966)

Auerbach et al. (1956, 1957 a) studied the occurrence of pathological changes in the bronchial epithelium in relation to smoking. The frequency of basal-cell hyperplasia, metaplastic changes and carcinoma in situ increased with the use of tobacco. The alterations were most pronounced in the patients who died of lung cancer Ford et al. (1961) found similar evidence, although they could not confirm that carcinoma in situ was more frequent in smokers. Chang (1957) showed that the occurrence of metaplasia and atypical epithelium was more frequent in smokers than in non-smokers. In a cytological material, the incidence of metaplastic changes and atypical metaplasia in the columnar epithelium was considerably higher in smokers than in non-smokers (Nasiell 1968 b)

It seems beyond doubt that there is a causal relationship between smoking and bronchogenic carcinoma (Watson & Conte 1954 Hackensellner 1957 a, Clemmesen 1961 Ministry of Health 1961) Other exogenous factors may however be a contributory cause for example inhalation of various chemical agents (Liebow 1952, Wilkins et al. 1957 Hackensellner 1957 a) It has been shown experimentally that in mice exposed to tobacco smoke hyperplastic alterations in the bronchial epithelium, with atypical changes, will develop (Leuchtenberger et al. 1958 Leuchtenberger et al. 1963) and that metaplastic changes and carcinomata of the respiratory tract may be produced in certain animals after oral administration of diethylnitrosamine (Dontenwill et al. 1961 a, 1961 b)

In a study of 54 cases of bronchogenic carcinoma (squamous-cell, 33 undifferentiated, 16 alveolar-cell, 3 and malignant cylindromata, 2) Auerbach et al. (1957 b) found carcinoma in situ or atypical changes in the bronchial epithelium in 48 cases. In 43 of 51 cases these alterations were also present in the opposite lung. The clinical significance is that cases which are regarded as recurrences or metastases are probably often carcinomata which have developed in the areas with premalignant changes. Carcinoma in situ or fully developed carcinoma of other locations in the bronchial tree were also reported by Black & Ackermann (1952), Ford et al. (1961) and Spencer (1968)

Mitotic Activity

In basal-cell hyperplasia, the mitotic activity in the bronchial epithelium is increased (Black

Chapter 5

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Through a stage of atypical epithelium carcinoma in situ develops. The atypical epithelium is hypercellular and consists of polymorphic cells with hyperchromatic nuclei and an irregular chromatin pattern. In carcinoma in situ the cellular atypia is more pronounced and occurs in all layers of the epithelium The cells are carcinoma-like but show no signs of invasive growth. Atypical epithelial changes and carcinoma in situ are almost exclusively found in metaplastic epithelium (Nasiell 1968 a, 1968 c)

It is presumed that the malignant neoplastic condition has a long preceding stage with morphological changes in the cells of the epithelium and that carcinoma represents the final stage. These antecedent epithelial changes may be diffuse while the invasive carcinoma involves only a limited area The precancerous changes in the bronchial epithelium are cellular atypia and carcinoma in situ preceded by metaplasia. However the extent to which metaplasia and precancerous cell atypia develop in parallel, or to which metaplasia precedes the atypical changes in the cells is unknown. It is generally believed that metaplasia occurs first, and metaplasia without atypical cells is therefore not regarded as a precancerous alteration (Nasiell 1968 c)

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Fig. 2.—Metaplastic bronchial epithelium (gallocyanin-chromalum, × 350)



Fig. 23.—Metaplastic bronchial epithelium (RNase gallocyanin-chromalum, × 350)

activity with a large formation of proteins (Caspersson 1947).

The present author has previously published a study of the concentrations of cytoplasmic RNA in metaplastic and morphologically normal bronchial mucosae (Greisen 1968).

Personal Investigations

Nuclear Measurements

The average content of DNA in metaplastic bronchial epithelium has in previous studies been related to that observed in normal diploid nuclei (Leuchtenberger et al. 1958, Bonani & Gandolfi 1961, Leuchtenberger et al. 1963). In comparison with normal epithelium, the average content of DNA in metaplastic epithelium was found to be increased. An explanation of this increase was not given. From tables in the two above-mentioned studies by Leuchtenberger et al. it is seen that there are both a greater dispersion of the values towards higher values,

& Ackermann 1952 Valentine 1957) Cunningham (1959) found that, in basal-cell hyperplasia actual mitoses were rare, but nuclei twice as large as normal were occasionally seen.

In animal experiments with H³-thymidine Dontenwill & Wiebecke (1964) observed considerably more labelled cells in metaplastic than in normal bronchial mucosae. The number of labelled cells increased with the degree of metaplasia In the fully developed carcinoma, the mitotic activity was of the same order as in the cases of severe metaplasia. The normal bronchi contained 0 85 ‰ labelled cells, but with increasing degrees of metaplasia the number rose to 15 48 69 and 58 ‰

In the study of tobacco-exposed mice, Leuchtenberger et al. (1958 1963) found no visible mitoses in the bronchial epithelium in a control group and only a few in a group with slight hyperplastic changes, whereas there were a larger number of mitoses in a group with more pronounced proliferative changes.

In experimental studies of the carcinogenic influence on the epidermis, the mitotic activity was found to increase from normal epidermis through various degrees of hyperplastic changes to the fully developed carcinoma (Kiljunen 1956 Dörmer et al 1964) The duration of DNA synthesis was of about the same order in normal skin hyperplastic skin and in carcinoma of the skin (Dörmer et al 1964)

Photometric Investigations

Boriani & Gandolfi (1961) studied the DNA content in human bronchi with varying degrees of metaplasia and found an average increase in the DNA content, especially in the basal nuclei, in parallel with the severity of the metaplasia. The distribution of the DNA values in the individual sections was not reported in the study

Microphotometric investigations of the bronchial mucosa in mice did not show any difference in the DNA content in the nuclei in morphologically normal mucosae exposed to cigarette smoke and a control group On the other hand it was observed that an average increase in the volume of the nuclei and an increase in the nuclear content of protein occurred in the cigarette-exposed animals before morphological changes developed in the epithelium. The average content of nuclear DNA increased with the degree of metaplasia (Leuchtenberger et al 1958 Leuchtenberger et al 1963).

Grundmann et al. (1961 a) made microphotometric measurements of the DNA content in the epithelial cells of the vaginal portion of the uterus both under normal conditions and through various degrees of epithelial changes to the fully developed carcinoma. In normal and slightly abnormal epithelium the DNA values were found mainly around a basal diploid value with some values in the range up to twice that figure.

The irregular and atypical epithelium revealed mainly tetraploid, or even higher values, whereas a still greater dispersion of the values was seen in the carcinoma In a comparison of DNA determinations performed on Feulgen-stained sections and nucleic acid determinations carried out on gallocyanin-chromalum-stained sections (Grundmann et al 1961 b) it appeared that the transition from normal to abnormal epithelium was accompanied by an increased RNA content of the basal cells. However in the study of these relatively thick sections, no regard was paid to cytoplasmic RNA which would be expected to increase with the atypia of the epithelium

As compared with normal the atypical epithelium revealed an increased cytoplasmic RNA content in the irregular and pre invasive stages (Grundmann et al 1960) Similar observations were made by Tsanev et al (1960) during the development of carcinoma of the skin. The cytoplasmic basophilia caused by RNA was found to be increased in precancerous conditions and in fully developed carcinomata of the skin as compared with normal epidermis.

The increased cytoplasmic content of RNA may be taken as evidence of increased cellular



Fig. 22.—Metaplastic bronchial epithelium (gallocyanin-chromalum, × 350).



Fig. 23.—Metaplastic bronchial epithelium (RNase, gallocyanin-chromalum, × 350).

activity with a large formation of proteins (Caspersson 1947).

The present author has previously published a study of the concentrations of cytoplasmic RNA in metaplastic and morphologically normal bronchial mucosae (Greisen 1968).

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Nuclear Measurements

The average content of DNA in metaplastic bronchial epithelium has in previous studies been related to that observed in normal diploid nuclei (Leuchtenberger et al. 1958, Boriani & Gandolfi 1961, Leuchtenberger et al. 1963). In comparison with normal epithelium, the average content of DNA in metaplastic epithelium was found to be increased. An explanation of this increase was not given. From tables in the two above-mentioned studies by Leuchtenberger et al. it is seen that there are both a greater dispersion of the values towards higher values,



Fig. 24.—Metaplastic bronchial epithelium (gallocyanin-chromalum, × 560).



Fig. 25.—Metaplastic bronchial epithelium (RNase gallocyanin chromalum, × 560).

and a displacement of the peak of the distribution curve towards higher values.

To investigate the distribution of the DNA values in metaplastic bronchial epithelium, the DNA content was measured in 30 nuclei. The large and dark nuclei with an obvious hyperdiploid content were excluded just as in the nuclear measurements on morphologically normal bronchial mucosae. As most of the cells exhibited this basal diploid value, it was not difficult to segregate the hyperdiploid cell group, even though considerably more hyperdiploid cells were present in metaplastic bronchial mucosae than in normal ones (see page 59).

Some sections contained not only metaplastic, but also morphologically normal epithelium (table 27).

From the table it appears that there is only a slight difference in the content of nucleic

Table 27 *Nucleic acid content in diploid nuclei.*

Section No.	Metaplastic epithelium DNA + RNA A.U	Metaplastic epithelium DNA A.U	Normal epithelium DNA + RNA A.U	Normal epithelium DNA A.U
37	13 75	12.5	12.4	
41	14.8	13.5		
44	14 1	12.1	13.6	
55	11 7	13 4	11.2	11.2
57	13 8	12 0	13 1	11.8
58	15 0	12.6		
61	12.5	10 8	13.0	
62	11.9	10 3		
Average	13 4	12 2	12.7	11.5



Fig. 26.—Distribution of DNA content in normal and hyperdiploid nuclei in metaplastic bronchial epithelium (section No. 44).

acid of diploid nuclei in the metaplastic and the normal epithelium in the same section. The difference in the nuclear content of DNA + RNA is smaller than reflected by the figures in the table, because the measurements of DNA + RNA were performed on non-RNase-treated sections in which the cytoplasmic RNA content will result in a greater extinction in metaplastic than in normal mucosae. However it will be difficult to avoid a slight uncertainty in the determination of the basal diploid value in metaplastic mucosae, with a tendency to a higher average value than normal, on account of the brisker mitotic activity

Measurements of the nuclear content of RNA will be difficult because the technique is beset with various limitations. The shape and orientation of the nuclei are not as regular as in normal bronchial epithelium, and the cytoplasmic extinction varies in the different layers of metaplastic mucosae. In addition, the determination of the basal diploid value in metaplastic mucosae is more difficult on account of the greater mitotic activity which tends to give a wider dispersion of the DNA values.

After the determination of the basal diploid value in the metaplastic epithelium, the number of hyperdiploid cells was counted. As in the morphologically normal bronchial mucosae, the limit for the hyperdiploid cells was fixed at 1½ × the mean of the diploid value. After the determination of the DNA content in all cell nuclei which appeared to be large and/or dark in comparison with the diploids, all nuclei with a calculated DNA content exceeding 1½ × the mean were put down as hyperdiploid. The number of hyperdiploid cells is stated per 500 cell nuclei. As distinct from the normal bronchial epithelium, in which no mitoses were seen, a few mitoses were found in the metaplastic epithelium. The DNA content in these nuclei was measured to be at about the tetraploid value. Figure 26 shows an example of the measurements of the DNA content in a metaplastic epithelium.

The number of hyperdiploid cell nuclei is stated in table 28

As already mentioned, the mitotic activity in metaplastic bronchial mucosae is increased. It is seen from figures 21 and 26 that the DNA content in the hyperdiploid cells is dis-

Table 28 *Hyperdiploid cell nuclei in metaplastic mucosae*

Section No.	DNA A.U	No. of cells counted	Hyperdiploid cells No.	Hyperdiploid cells per 100 cells
37	12.5	500	14	2.8
41	13.5	500	13	2.6
44	12.[illegible]	500	16	3.2
55	13 4	500	20	4.0
57	1 0	500	19	3.8
58	1 6	500	21	4.2
61	10 8	500	16	3.2
6[illegible]	10 3	500	6	1.2
Average				3.1

tributed in the range from the basal diploid value to twice that figure One cell nucleus was found with a DNA content twice the tetraploid value It was a nucleus at prophase with incipient condensation of the nuclear chromatin (section No 55 atypical metaplasia) In all other sections the values of the nuclei were up to but not higher than twice the diploid value and might be accepted as cells which were synthetizing DNA in preparation for mitosis. The mitotic activity observed in the metaplastic epithelium is in good agreement with the results of the investigations with radioactive thymidine in metaplastic bronchial epithelium (Dörmer et al 1964) However there is no conclusive proof that all cells with a hyperdiploid content of DNA are synthetizing DNA Some of the hyperdiploid cells might have an aneuploid content of DNA

The distribution curve for the hyperdiploid cells is nevertheless in favour of the assumption that the cell nuclei are synthetizing DNA If the DNA content just as in the hyperdiploid nuclei in normal bronchial mucosae, is related to a mean of 12 A U and the distribution is plotted for the hyperdiploid nuclei the resulting curve will be similar to the distribution curve for the hyperdiploid nuclei in morphologically normal mucosae (the octaploid cell nucleus which was found in one section with atypical metaplasia is included as two tetraploid nuclei) (figs. 20 and 21)

It can be seen from the distribution curve (fig. 21) that the mitotic activity in the metaplastic epithelium just as in normal bronchial epithelium is about 55 % larger than the number of hyperdiploid cell nuclei suggests. The number of all synthetizing cells in the metaplastic mucosae is thus, on an average, about 4 8 %

If all the hyperdiploid cells observed can be taken as cells synthetizing DNA the mitotic activity in metaplastic mucosae will be 4 8 % as against 0 48 % in morphologically normal bronchial mucosae i e the mitotic activity is 10 times as large as in normal bronchial epithelium.

The metaplastic epithelium showed basal-cell hyperplasia (sections Nos. 37 41 44) simple metaplasia (sections Nos. 58 61 62) and atypical metaplasia (sections Nos. 55 57).

The average percentages of hyperdiploid cells were 2 9 % 2 9 % and 3 9 % respectively The material is too small to assess the number of hyperdiploid cells in the various degrees of metaplasia

Cytoplasmic Measurements

In the evaluation of the RNA concentration in metaplastic bronchial epithelium the cytoplasmic extinction was measured (1) around the basal cells, (2) in the intermediate layer and (3) in the cytoplasm of the superficial cell layer For each of the three layers, the cytoplasmic extinction is stated as the average of 50 measurements.

Before the measurements on the cytoplasm were performed the content of mucopolysaccharides, if any was assessed by PAS staining. Only in a single section with basal-cell hyperplasia were there so many mucin-secreting cells in the superficial cell layer that the cytoplasmic content of RNA could not be assessed with certainty

After treatment with RNase the cytoplasmic extinction was measured in the basal, intermediate and superficial cell layers. Each figure for the RNase-treated sections represents the average of 15 measurements In the determination of the cytoplasmic extinction it is the concentration of RNA which is measured The volume of the cytoplasm is, however most likely larger in metaplastic epithelium than in the basal cells of morphologically normal bronchial epithelium It therefore appears certain that there is an increase not only in the concentration of RNA but also in its content in metaplastic epithelium (figs. 4 8 9 22 24)

The series of metaplastic mucosae studied consisted of eight biopsy specimens from the original material supplemented by nine biopsy specimens from the Institute of Pathology (table 29)

After treatment with RNase the intense cy

Table 29 *Cytoplasmic extinction in metaplastic epithelium*

Section No.	Basal	Intermediate	Superficial	Average
37	0 29	0.29	0.29	0.29
41	0 32	0 30	0 26	0 29
44	0 17	0.22	0.23	0.21
55	0 23	0.22	0.21	0.22
57	0 22	0.23	0.25	0.23
58	0 27	0.28	0.24	0.26
61	0 24	0 26	0.20	0.24
62	0.25	0 28	0.27	0.26
L 1	0 22	0 24	0 21	0.22
L 2	0 24	0.17	0.22	0.21
L 3	0.29	0 29	0.27	0.28
L 4	0 19	0 26	–	0 23
L 5	0 27	0 28	0 31	0 29
L 9	0 39	0 4..	0 36	0.39
L 10	0 26	0 27	0.26	0..6
L 11	0.23	0 23	0.26	0.23
L 14	0 31	0 32	0.34	0.32
Average				0 26

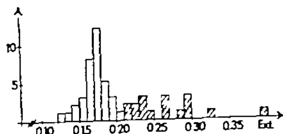


Fig. ?7.—Distribution of the average cytoplasmic RNA concentration in metaplastic bronchial epithelium (hatched columns) and in normal bronchial epithelium (white columns).

toplasmic basophilia disappeared completely evidencing that the substance observed was RNA (table 30)

It is seen that there was a considerably increased concentration of RNA in the cytoplasm of the metaplastic mucosae, as compared with the morphologically normal ones.

Table 30. *Cytoplasmic extinction in RNase treated metaplastic bronchial epithelium.*

Section No	Basal	Intermediate	Superficial	Average
37	0 05	0 04	0 04	0 04
41	0 04	0 04	0 04	0 04
44	0 04	0 04	0 04	0 04
55	0 04	0 04	0 04	0 04
57	0 05	0 04	0 05	0 04
58	0 04	0 05	0 03	0 04
61	0 02	0 02	0 02	0 02
62	0 03	0 04	0.04	0 03
L 1	0 03	0 03	0 04	0 04
L	0 02	0 03	0.04	0 03
L 3	0 02	0 03	0 03	0 02
L 4	0 02	0 0^		0 02
L 5	0 03	0 04	0 04	0 03
L 9	0 03	0 03	0 03	0 03
L 10	0 03	0 02	0 03	0 03
L 11	0 03	0 04	0 04	0 04
L 14	0 02	0 03	0 03	0 03
A erage				0 03

The average for the entire material of metaplastic epithelium was 0.26, as against 0 17 in the morphologically normal mucosae. Figure 27 shows the concentration of RNA in metaplastic and normal mucosae.

In eight sections, the epithelium showed basal-cell hyperplasia, in six cases simple metaplasia, and in three atypical metaplasia. The average concentrations of RNA in the three groups were 0.26, 0.25 and 0.28 respectively The differences are not significant.

Conclusions

The metaplastic bronchial mucosae differ from the normal ones with regard to the nucleic acid content in nuclei and cytoplasm.

Like normal epithelium, the metaplastic mucosae contain a population of cells with diploid nuclei, but, as compared with the normal ones, they also have a considerably increased number of hyperdiploid nuclei, viz. 10 times as many This is presumably due to cells which are synthetizing DNA in preparation for mitosis, even though it cannot be excluded that some of the cells may have an increased aneuploid DNA content. The nuclear content of DNA does not show as might be expected from the results of previous investigations, a gradual increase in the DNA content of the cell nuclei. In the metaplastic mucosae there is, as in the normal mucosae, not only a basal diploid stem cell, but also an increased number of cell nuclei with a hyperdiploid DNA content.

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61	0.24	0.26	0.20	0.24
62	0.25	0.28	0.27	0.26
L 1	0.22	0.24	0.21	0.22
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L 4	0.19	0.26		0.23
L 5	0.27	0.28	0.31	0.29
L 9	0.39	0.42	0.36	0.39
L 10	0.26	0.27	0.26	0.26
L 11	0.21	0.23	0.26	0.23
L 14	0.35	0.32	0.34	0.3
Average				0.26

toplasmic basophilia disappeared completely evidencing that the substance observed was RNA (table 30).

It is seen that there was a considerably increased concentration of RNA in the cytoplasm of the metaplastic mucosae, as compared with the morphologically normal ones.

Table 30 *Cytoplasmic extinction in RNase-treated metaplastic bronchial epithelium*

Section No	Basal	Intermediate	Superficial	Average
37	0.05	0.04	0.04	0.04
41	0.04	0.04	0.04	0.04
44	0.04	0.04	0.04	0.04
55	0.04	0.04	0.04	0.04
57	0.05	0.04	0.05	0.04
58	0.04	0.05	0.03	0.04
61	0.02	0.02	0.02	0.02
62	0.03	0.04	0.04	0.03
L 1	0.03	0.03	0.04	0.04
L 2	0.02	0.03	0.04	0.03
L 3	0.02	0.03	0.03	0.02
L 4	0.02	0.02		0.02
L 5	0.03	0.04	0.04	0.03
L 9	0.03	0.03	0.03	0.03
L 10	0.03	0.02	0.03	0.03
L 11	0.03	0.04	0.04	0.04
L 14	0.02	0.03	0.03	0.03
Average				0.03

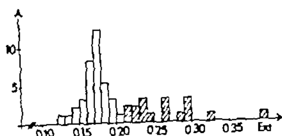


Fig. 27.—Distribution of the average cytoplasmic RNA concentration in metaplastic bronchial epithelium (hatched columns) and in normal bronchial epithelium (white columns).

The average for the entire material of metaplastic epithelium was 0.26 as against 0.17 in the morphologically normal mucosae. Figure 27 shows the concentration of RNA in metaplastic and normal mucosae.

In eight sections, the epithelium showed *basal-cell hyperplasia*, in six cases simple metaplasia, and in three atypical metaplasia. The average concentrations of RNA in the three groups were 0.26, 0.25 and 0.28 respectively. The differences are *not significant*.

Conclusions

The metaplastic bronchial mucosae differ from the normal ones with regard to the nucleic acid content in nuclei and cytoplasm.

Like normal epithelium, the metaplastic mucosae contain a population of cells with diploid nuclei, but, as compared with the normal ones, they also have a considerably increased number of *hyperdiploid nuclei*, viz. 10 times as many. This is presumably due to cells which are synthetizing DNA in preparation for mitosis, even though it cannot be excluded that some of the cells may have an increased aneuploid DNA content. The nuclear content of DNA does not show as might be expected from the results of previous investigations, a gradual increase in the DNA content of the cell nuclei. In the metaplastic mucosae there is, as in the *normal* mucosae, not only a basal diploid stem cell, but also an increased number of cell nuclei with a hyperdiploid DNA content.

The cytoplasmic concentration and most likely also the cytoplasmic content of RNA are considerably increased in comparison with the basal cells of morphologically normal bronchial epithelium

It appears that only when morphological alterations in the form of metaplasia have occurred in the bronchial epithelium can changes in its nucleic acid content be demonstrated, viz. an increase both in the number of hyper diploid cells and in the concentration of RNA in the cytoplasm

BRONCHIAL ADENOMA

Bronchial adenoma covers two different types of tumours, viz. the carcinoid tumour and the cylindroma, which differ in histological structure and secretory properties.

The carcinoid tumours comprise 90 % of the adenomata, while the remaining 10 % are cylindromata. The tumours are nearly always localized in the large bronchi, and 90 % of them can be seen through the bronchoscope. The adenoma is equally frequent in the two sexes. It occurs in all age groups, even in children (Kreyberg 1962, 1967). The average age of the patients is within the range of 44–48 years.

The cylindromata arise from the mucous glands in the bronchial mucosa. This is evidenced by their resemblance with other mucous-gland tumours, the production of mucin, and the absence of these tumours in the periphery where the bronchial mucosa does not contain glands. The origin of the carcinoid tumours is more disputable, and many theories of their origin have been advanced. Histologically they have many features in common with the tumours of the same name in the digestive tract, and, like the latter, they probably arise from argentaffine cells in the epithelium or from neurosecretory cells in the mucous glands (Spencer 1962, 1968). Engelbreth-Holm (1944) expressed the view that there was no reason to segregate a special group of adenomata as cylindromata, as cylindroid portions may develop both in bronchial adenomata and in various other epithelial tumours. Clinically the carcinoid syndrome may be seen in bronchial carcinoid tumours as well as in the carcinoids of the intestine (Weiss et al. 1961).

The adenoma is growing either as a bronchial polyp or as an infiltrating submucous tumour (Spencer 1962).

Histologically the carcinoid tumour is composed of small, uniform cells with regularly rounded or slightly oval nuclei with evenly distributed fine chromatin granules. The cells grow in solid cords, in trabeculae, or show a glandlike structure. Mitoses are rarely seen (Liebow 1952, Kreyberg 1962, Spencer 1962).

The cylindromata are, although to a different extent, mucin-producing. Most commonly the shape of the cell does not differ from that of the normal gland cells, but there may be various degrees of anaplasia. The cells grow in trabeculae or tubes, which may contain PAS-positive mucin. Mitoses are here more frequent than in the carcinoid tumours.

The adenoma is usually a benign tumour. It grows slowly and spreads mainly by local invasion. Lymphogenic metastases occur in less than one half of the cases and are usually late. In less than 2 % there is haematogenic extension (Liebow 1952, Kreyberg 1962, Spencer 1962).

Nucleic Acid Determinations

Only a few studies of the DNA content and chromosomes in benign tumours are available.

Stich et al. (1960) investigated the DNA content in normal epithelium, in histologically benign polyps and in adenocarcinomata of the large intestine. No polyploid cell nuclei were found in the normal epithelium of the intestine, i. e. no cell nuclei with a DNA content higher than twice the basal diploid value. Among eight histologically benign polyps, two revealed metaphases with a DNA content twice as large as that of the metaphases in the diploid class,

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i. e. the cells had a modal value within the tetraploid range. In all the remaining cases, the *modal value was within the diploid range*, although it was a little higher than the basal somatic diploid value in the lymphocytes. As distinct from the wide dispersion of the values in the adenocarcinoma due to an aneuploid number of chromosomes there was only a slight dispersion of the metaphase values in the polyps.

Jacobsen (1968) studied the nucleic acid content in benign prostatic adenomata and found a slightly increased average content of DNA + RNA as compared with nuclei in normal prostatic epithelium. The dispersion around the *mean was greater than in the normal nuclei* of the prostate. In terms of arbitrary units, the nucleic acid content was 9.1 A. U. in normal nuclei, and 10.5 A. U. in the adenomata.

In microphotometric studies of mammary tumours, Emson et al. (1967) found a DNA content of only up to twice the diploid value in cystic hyperplasia. In the intraductal carcinoma with uniform cell types, values up to the tetraploid value were also found while a wider dispersion with values above the tetraploid value was revealed in the comedo carcinoma. In the adenocarcinoma, the DNA content showed a wide dispersion, with many values above the tetraploid level.

Similar observations were made by Bader et al. (1960) in a study of ovarian tumours. In benign tumours, DNA values above twice the basal value were not found, and the determinations of the DNA content indicated that mitotic irregularities were either absent or of very slight importance for the DNA content of benign and slightly malignant tumours.

In benign tumours of the thyroid, Garneau (1964) found a modal value at the diploid level determined by a comparison with the DNA content of lymphocytes.

In chromosome studies of benign tumours a normal diploid number of chromosomes was observed in virus induced papillomata. On the other hand after malignant transformation the carcinomata which developed were characterized by a hyperdiploid modal number of chromosomes (Hsu 1961). Palmer (1959) likewise reported that most cells in virus induced papillomata had a diploid number of chromosomes.

Chromosome studies on adenomata of the colon revealed some pseudodiploid nuclei and many aneuploid metaphases, yet with only small deviations from normal values. Most metaphases were hyperdiploid. Abnormal individual chromosomes were rare in the adenomata (Enterline et al. 1967).

In a group of mammary tumours, chromosome studies showed normal diploid metaphases in benign cystic hyperplasia, while the cells in carcinomata revealed a diploid modal number in two cases, a triploid in five, and a pentaploid number of chromosomes in one (Toews et al. 1968).

Only a few investigations of the nucleic acid content of bronchial adenomata are available. In a series of biopsy specimens from the bronchi Boriani & Gandolfi (1961) made microphotometric measurements of the DNA content of carcinoid tumours after Feulgen staining. The number of adenomata studied was not stated. The average content of DNA in the adenomata was a little higher than in the nuclei of normal bronchial epithelium. The histograms are narrow with only a slight dispersion, and the values are grouped symmetrically around one value. The adenomata differed clearly from the malignant bronchial neoplasms not only in the smaller average content of DNA in the nuclei but also in the very slight dispersion of the values.

Seidel & Sandritter (1963) studied microphotometrically the DNA content in a cystic lung adenoma and a case of malignant lung adenomatosis (alveolar-cell carcinoma). The distribution of the nuclear DNA values in the cystic lung adenoma showed a maximum at the diploid value. The values were dispersed up to, but not above the tetraploid level. Cells with these values were probably in the premitotic stage synthetizing DNA.

In the cytoplasm of papillomata of the

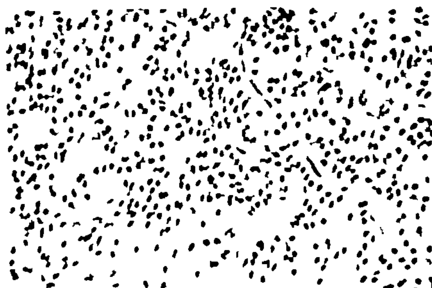


Fig. 21.—Bronchial adenoma (carcinoid) (gallocyanin-chromalom, 350)

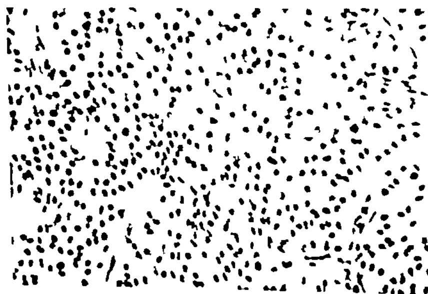


Fig. 22.—Bronchial adenoma (carcinoid) (RNase, gallocyanin-chromalom, 350)

bladder Caspersson & Santesson (1942) found an increasing concentration of cytoplasmic RNA from benign through more malignant to fully developed carcinoma cells. No investigations of the cytoplasmic concentration of RNA in bronchial adenomata or other benign tumours seem to be on record.

Mitoses

In microscopic studies of tissue from bronchial adenomata, none or only a few mitoses are found in the carcinoid tumours, while mitoses are more frequent in the cylindroma (Engelbreth-Holm 1944, Liebow 1952, Kreyberg 1962, Spencer 1962).

No reports on the mitotic activity in bronchial adenomata exist, and on the whole only a few studies of the mitotic activity in benign tumours are available.

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Fig. 28.—Bronchial adenoma (carcinoid) (gallocyanin-chromalum, × 350).

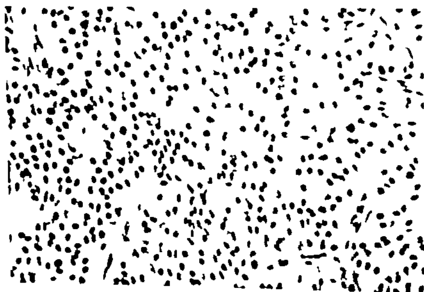


Fig. 29.—Bronchial adenoma (carcinoid) (RNase, gallocyanin-chromalum, × 350).

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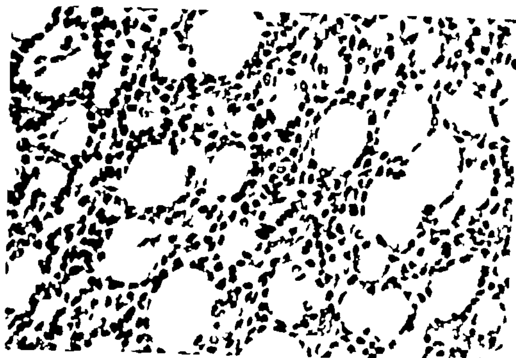


Fig. 30 —Bronchial adenoma (cylindroma) (gallocyanin-chromalum, × 350).



Fig. 31 —Bronchial adenoma (cylindroma) (RNase gallocyanin-chromalum × 350).

benign ovarian cystadenoma was of the same order as in normal skin while it was less pronounced in a scirrhous mammary tumour from the same patient.

That the mitotic activity does not directly depend on the degree of malignancy of the tumour appears from a study of mammary cancer published by Johnson et al in 1961 The mitotic activity was of the same order in a normal duct and in carcinoma cells, whereas it was three times as high in a fibro-adenoma

Personal Investigations

The material studied consisted of 14 biopsy specimens of bronchial adenomata secured from eight patients. The sections A 2, A 3 and A 16 are from different sites of the same tumour whereas sections A 9 A 10 A 11 A 13 and A 17 are all cylindromata originating from the same patient. The biopsies were performed at intervals of a few weeks or months.

Cylindromata are usually more malignant

Table 31 *Nucleic acid content in diploid nuclei of bronchial adenomata.*

Section No.	DNA + RNA A. U	DNA A. U.
A 1	11.7	11.6
A 2	14.5	13.5
A 3	9.8	9.2
A 16	10.8	9.3
A 4	16.1	15.8
A 7	10.6	9.1
A 8	12.5	11.3
A 9	12.8	11.0
A 10	11.2	10.8
A 11	11.0	10.4
A 13	14.0	13.7
A 17	11.1	10.7
A 14	13.5	13.3
A 15	9.7	9.0
Average	12.1	11.3

than carcinoid tumours. The cylindromata in this material all revealed a benign histological structure and the clinical course was also benign.

Nuclear Measurements

Determination of the nucleic acid content in the nuclei was performed in the same way as in the normal bronchial mucosae. In each of the non-RNase-treated sections, the nucleic acid content was measured in 30 nuclei. In the RNase-treated sections, in 20 nuclei. The measurements of the total nuclear nucleic acid content included a small amount of cytoplasmic RNA (table 31).

The total nuclear nucleic acid content averaged 12.1 A. U (as compared with 12.6 A. U in the normal epithelial nuclei). The dispersion of the means in the sections (from 9.7 to 16.1 A. U.) is a little greater than in the normal epithelium, which may probably be ascribed to the more unequal treatment of these biopsy specimens (fig. 32).

The average DNA content in the nuclei is of the same order as in the normal nuclei (see page 34).

The modal value of the bronchial adenomata was determined as follows: The DNA content of the tumour stem cell was related to that of the normal diploid nuclei in the section,

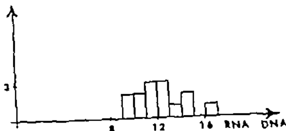


Fig. 32.—Distribution of the average nuclear nucleic acid content in bronchial adenomata.

i.e. in nuclei of normal bronchial epithelium, if such were present, or in lymphocytes, which are nearly always present in the sections (Ricards *et al.* 1956, Atkin & Richards 1956, Bader 1959 Stich et al. 1960, Seidel & Sandritter 1963 Garneau 1964 Atkin et al. 1966, Emson & Kirk 1967).

In photometric measurements, it is possible to determine the modal value with reasonable certainty in nearly all tumours. Many nuclei are grouped around a basal value, sometimes with a distinct secondary accumulation of results at twice or perhaps, four times the basal value (Atkin *et al.* 1966).

The position of the modal value is stated in relation to the diploid or near-diploid, triploid or near-triploid, and tetraploid or near tetraploid values. The limits were fixed so that the ratios between modal and diploid values were in the range from 0.75 to 1.25 from 1.25 to 1.75 and from 1.75 to 2.25 respectively (Wakonig Vaartaja et al. 1967) (table 32).

In agreement with previous studies on modal values in benign tumours, it appeared that the modal values in nearly all bronchial adenomata were within the diploid or near-diploid range. Only in one case was it within the *near-triploid range* (section No. A 4).

The number of hyperdiploid nuclei in the bronchial adenomata was determined in the same way as in the normal and the metaplastic bronchial epithelium (fig. 33).

The number of hyperdiploid nuclei is stated per 1000 tumour nuclei (table 33).

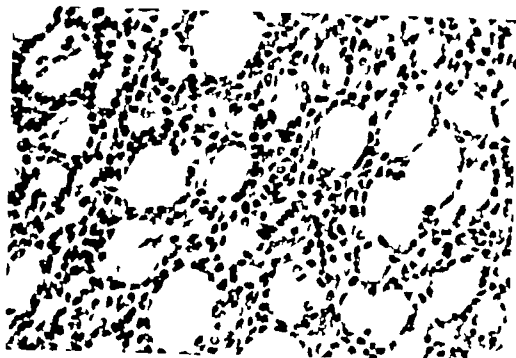


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Personal Investigations

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Cylindromata are usually more malignant

Table 34 *Cytoplasmic RNA concentration in bronchial adenomata.*

Section No	Non-RNase-treated		RNase-treated	
	Adenomata	Connective tissue	Adenomata	Connective tissue
A 1	0.19	0.04	0.05	0.04
A 2	0.16	–	0.03	–
A 3	0.15	0.05	0.03	0.02
A 16	0.15	0.04	0.04	0.02
A 4	0.18	0.04	0.04	0.03
A 7	0.16	0.05	0.03	0.03
A 8	0.14	0.04	0.04	0.03
A 9	0.12	0.04	0.04	0.03
A 10	0.19	0.05	0.04	0.03
A 11	0.15	0.03	0.04	0.03
A 13	0.30	0.03	0.04	0.03
A 17	0.18	–	0.03	–
A 14	0.19	0.05	0.03	–
A 15	0.17	0.03	0.04	0.04
Average	0.17	0.04	0.04	0.03

of the cytoplasmic RNA concentrations (table 34).

From table 34 it appears that the average cytoplasmic extinction is of the same order as in normal bronchial epithelium. There is a certain variation in the values around the mean. Only in one case a cylindroma, was a distinctly increased concentration of cytoplasmic RNA, 0.30 revealed, while the other cylindromata did not show any difference as compared with the carcinoid tumours. The distribution of the average cytoplasmic extinction appears from figure 34



Fig 34—Distribution of the average cytoplasmic RNA concentration in bronchial adenomata.

Conclusions

The average values of the nuclear nucleic acid content in the adenomata were of the same order as in normal epithelial nuclei. The modal value was in just one case within the triploid range, while in all other cases, they were within the diploid or near-diploid range. The number of hyperdiploid cell nuclei was, probably as an expression of mitotic activity three times as large as in normal bronchial epithelium. With one exception, the extinction of the cytoplasm was as in normal bronchial epithelium.

The adenomata thus differ from normal bronchial epithelium in having a larger number of hyperdiploid cells. An elevated modal value was observed in one case and an increased cytoplasmic RNA concentration in one. On the other hand, the adenomata differ distinctly from carcinomata in that there is only a small dispersion of the values of the nuclear nucleic acid content, and the DNA content does not exceed twice the basal modal value.

Table 32 *Modal values for the bronchial adenomata*

Section No	Modal value A. U	Diploid value A. U	Modal/diploid ratio
A 1	11 6	11 1	1 05
A 2	13.5	–	–
A 3	9.2	9 0	1 02
A 16	9.3	8.5	1 09
A 4	15 8	11 7	1.35
A 7	9 1	9 9	0 92
A 8	11.3	11 8	0 96
A 9	11 0	11 4	0.96
A 10	10 8	–	–
A 11	10 4	10.2	1 0
A 13	13 7	11.9	1 15
A 17	10 7	9.3	1 15
A 14	13.3	–	–
A 15	9 0	–	–

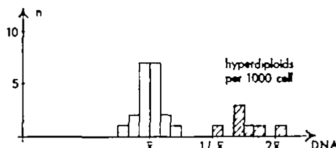


Fig. 33 —Distribution of DNA content in normal and hyperdiploid nuclei in bronchial adenoma (section No A 10)

Table 33 *Number of hyperdiploid nuclei in the bronchial adenomata.*

Section No	No of cells counted	Hyperdiploid nuclei	
		No	per 1000
A 1	1000	17	17
A 2	1000	9	9
A 3	1000	26	6
A 16	1000	21	1
A 4	1000		
A 7	1000	7	7
A 8	1000	19	19
A 9	1000	1	3
A 10	1000	7	7
A 11	1000	4	4
A 13	1000	10	10
A 17	1000	3	1
A 14	1000	19	19
A 15	1000	1	1
Average			11 4

It appears that the average number of hyperdiploid nuclei in the bronchial adenomata is about three times as large as in the normal bronchial epithelium and about three times as low as in the metaplastic mucosa.

It is likely that the hyperdiploids are synthetizing DNA in preparation for mitosis. No nuclei were found in the range from above twice the modal value, and most nuclei were around the basal modal value. In the microphotometric investigations, however, it could not be excluded that some nuclei with a hyperdiploid DNA content might be aneuploid.

From table 33 it appears that there may be quite an appreciable variation in the number of hyperdiploid nuclei in various places of the same tumour (sections Nos. A 2, A 3, A 16). A repetition of the measurements gave the same results.

It is also seen that the average percentage of hyperdiploid nuclei was 0 54 in the cylindromata as against 1 47 in the carcinoid tumours.

As, histologically, the cylindroma usually shows more malignant features than the carcinoid tumours, a larger number of hyperdiploid cell nuclei might be expected in the former. However, the histological structure of the cylindromata in the present series and the clinical course were benign, and the material is too small to allow a statistical comparison.

Cytoplasmic Measurements

The measurements of the cytoplasmic RNA concentrations in the adenomata were, as in the other tissues, performed at 570 nm. The results are calculated as an average of 50 measurements performed in one or two places per cell, depending on the density of the cells. Measurements of the extinction were also carried out on connective tissue of the tumours and, in the RNase treated sections, on the cytoplasm of the tumour cells and the connective tissue of the tumours. A few adenomata contained scattered PAS-positive material. These places were avoided during the determination

with about the same frequency centrally (36 %) and peripherally (33 %)

The growth of squamous-cell carcinoma may be relatively slow and metastases are often confined to regional lymph nodes. The development of small-celled anaplastic carcinoma is more rapid with a greater tendency to distant metastases, which are not infrequently the presenting symptom (Kreyberg 1962). Slowly growing tumours often have a higher rate of operability and a better prognosis than rapidly proliferating and rapidly metastasizing tumours (Andersen et al. 1969)

Lymph-node metastases occur more often in the small-celled anaplastic carcinoma than in the two other types (Nohl 1962, Jepsen 1966)

A considerably greater extrapulmonary dissemination of tumour cells was also reported by Maamies (1966) in cases of small-celled anaplastic carcinoma as distinct from squamous-cell carcinoma and adenocarcinoma (41.3 % 26.1 % and 0.5 % respectively) This dissemination is associated with a grave prognosis (Watson 1958, Nohl 1962, Hukill et al. 1962)

Growth into the large pulmonary vessels, especially *the veins,* is a very grave prognostic sign (Collier et al. 1957 Spencer 1968)

The size and the localization of the tumour are also assumed to be of substantial importance in the prognosis. Most of the small tumours are central, and will produce symptoms at an early stage, while the larger tumours are mostly found peripherally indicating that the symptoms occur at a later stage in the development of the disease (Walter & Pryce 1955 b). The fact that so many bronchogenic tumours are peripheral contributes to the poor prognosis (Walter & Pryce 1955 b) On the other hand, peripheral tumours are more often operable than the central ones, which is a good sign as far as the prognosis is concerned (Andersen et al. 1969)

Various studies have shown that the prognosis is most favourable for squamous-cell carcinoma, just as good or nearly so for adenocarcinoma, while it is much graver for small-celled anaplastic carcinoma (Kirklin et al. 1955 Boucot et al 1959 Watson & Berg 1962, Goldman 1965 Hyde et al. 1965 Maamies 1966 Jones et al. 1967)

Many factors are thus of importance in the prognosis of bronchogenic carcinoma, the histological type of tumour its localization, its tendency to haematogenic or lymphogenic spread, its growth into blood vessels, and its growth rate.

As far as bronchogenic carcinomata are concerned, it has also been revealed that the type of carcinoma with the best prognosis contains the largest number of polyploid cell nuclei (Greisen 1969)

Nucleic Acid Content

The nucleus of the cancer cell often differs from that of the normal cell nuclei in shape, size and staining capacity

The shape and the size are more variable than in normal cell nuclei. The shape is often more irregular and the nucleus of the cancer cell is usually larger than that of the normal cell. *None of these changes are, however specific* for the cancer cell (Oberling & Bernhard 1961)

Caspersson & Santesson (1942) described two different types of cancer cells. In type A, the nucleus is of normal or slightly increased size. The *nucleus* contains a large amount of DNA, and there is a distinct, but not particularly large, *nucleolus*. Type-A cells are mainly found peripherally in the tumour tissue, around the vessels and infiltrating the connective tissue, where the most favourable nutritive conditions exist. In type B, the nucleus is considerably larger with a smaller content of DNA, and with a large nucleolus containing a large *amount of* RNA. This cell type is found mainly in the central part of a tumour the more central the cell is, the more typical it becomes. From type B there is a gradual transition to necrotic cells, which contain a still smaller amount of DNA.

By chemical determinations of the content of nucleic acids in a number of tumours, Wüst

Chapter 7

BRONCHOGENIC CARCINOMA

Pathological Anatomy

The three most common and best defined bronchogenic carcinomata are the squamous-cell carcinoma, the small-celled anaplastic carcinoma and the adenocarcinoma, mentioned in the order of decreasing frequency. The large-celled anaplastic carcinomata are not so well defined; most of these can by various histochemical procedures, be grouped either under squamous-cell carcinomata or particularly under adenocarcinomata (Kreyberg 1962, Maamies 1966).

Epidermoid or squamous-cell carcinoma is the designation for the type which shows stratification of the cells, intercellular bridges, wheel formation or keratinization (Kreyberg 1962, Spencer 1968).

The small-celled anaplastic carcinoma is characterized by small spheric to oval cells with very sparse cytoplasm. The cells are usually grouped in clusters or cords without stratification. Many mitoses are often seen. The basal, morphological criterion for the adenocarcinoma is the glandular structure with or without mucin.

The use of tobacco, especially cigarette smoking, and other exogenous factors are important causal factors in the development of epidermoid carcinoma and small-celled anaplastic carcinoma, whereas the frequency of the adenocarcinoma is not significantly increased by excessive smoking (Liebow 1952, Kreyberg 1962).

Bronchogenic carcinoma rarely occurs before the age of 30–39 years, and its frequency increases steeply with advancing age.

In most cases, especially as far as squamous-cell and small-celled anaplastic carcinomata are concerned, the surface epithelium seems to be the site of origin of the tumour. Presumably adenocarcinoma also arises from the surface epithelium (Jonas & Hukill 1968). Gradual transition from atypical stratified squamous epithelium to carcinoma in situ and manifest carcinoma may occur (Liebow 1952). It is usually assumed that most bronchogenic carcinomata arise from the epithelium covering the bronchi and bronchioles. Probably they develop from the basal cells, which are able to differentiate into cells which differ in function and structure (Walter & Pryce 1955 a).

Squamous-cell carcinoma arises from foci of squamous-cell metaplasia, adenocarcinoma from atypical cylindrical cells or from mucous glands, while small-celled anaplastic carcinoma is presumed to develop from basal cells of the bronchial epithelium (Watson & Berg 1962, Spencer 1968).

One third of the squamous-cell carcinomata, three quarters of the adenocarcinomata and one fifth of the small-celled anaplastic carcinomata arise in the periphery of the bronchial system (Spencer 1968). Maamies (1966) found that of squamous-cell carcinomata 63 % were central, 22 % intermediate and 15 % peripheral. The corresponding figures for adenocarcinomata were 44 %, 30 % and 25 %, and for small-celled anaplastic carcinoma 74 %, 20 % and 6 % respectively. In a group of resected tumours, Walter & Pryce (1955 b) found that squamous-cell carcinoma occurred more frequently centrally (44 %) than peripherally (28 %), while adenocarcinoma was most commonly localized peripherally (84 %) and that small-celled anaplastic carcinoma occurred

with about the same frequency centrally (36 %) and peripherally (33 %)

The growth of squamous-cell carcinoma may be relatively slow and metastases are often confined to regional lymph nodes. The development of small-celled anaplastic carcinoma is more rapid with a greater tendency to distant metastases, which are not infrequently the presenting symptom (Kreyberg 1962) Slowly growing tumours often have a higher rate of operability and a better prognosis than rapidly proliferating and rapidly metastasizing tumours (Andersen et al. 1969)

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(Blumenfeld 1943 Johnson et al. 1960 Johnson et al. 1961). In certain normal tissues, for example, the intestinal epithelium, the mitotic activity is greater than in many tumours (Bertalanffy & Lau 1962 b)

However during carcinogenesis, the mitotic activity is often seen to increase from normal tissue through precancerous alterations to fully developed carcinoma (see page 56)

Whereas only a few cells with DNA values above the diploid value are revealed by photometric studies of normal tissues, many cells with a DNA content above that value will usually be found in cell populations of malignant tumour tissue (Atkin & Richards 1956, Sandritter, Müller et al. 1958). In various bronchogenic carcinomata, Sandritter Müller et al. (1958) thus found 50–90 % of the cell nuclei with DNA values higher than 1 /₂ × the modal value.

Numerous biochemical and microphotometrical studies of the DNA content of tumour tissue in animals and in man are on record, both in spontaneous, induced and transplanted tumours.

In biochemical studies, the average DNA value of the tumour-cell nuclei is determined by relating the DNA determinations to the number of tumour-cell nuclei. Of greater interest are microphotometric investigations, in which determination of the DNA content in individual nuclei can be performed in addition to measurements of the average DNA content of the tumour nuclei. In this way an expression of the distribution of the DNA values in the tumour tissue concerned is obtained. Good agreement between biochemical and microphotometric determinations of the average DNA value of cell nuclei was found by Leuchtenberger et al. (1952 a, 1952 b)

Microphotometric determinations of the DNA content has been performed in tumours from various human organs, especially the urogenital system, the skin and the mammary gland (Stowell & Cooper 1945 Stowell 1947 Sandritter 1952 a, 1952 b, Leuchtenberger et al. 1954 b, Atkin & Richards 1956, Grundmann et al. 1960 Atkin & Richards 1962, Sandritter & Fischer 1962, Laumonier et al. 1963 Atkin 1964 a, 1964 b Jacobsen 1968)

DNA Content of Bronchogenic Carcinomata

Microphotometric studies of bronchogenic carcinomata have mostly been sporadic investigations performed in connection with determination of the DNA content in tumours from various organs.

Among many other tumours, Stowell (1946) studied two bronchial adenocarcinomata. The average DNA content was 1.53 and 1 86 times, respectively as great as that of normal bronchial epithelial nuclei.

Among 19 carcinomata, Sandritter (1952 a) studied three squamous-cell carcinomata and five small-celled bronchial carcinomata (autopsy material). In terms of arbitrary units, the DNA content in squamous-cell carcinomata varied from 1 98 to 2.76, and the RNA content per cell, i. e. RNA in nucleus plus cytoplasm, from 1 43 to 1 8. In comparison, the small-celled bronchial carcinomata had DNA values within the range from 1.36 to 1.83 and a total RNA content per cell from 0 16 to 0.53

Leuchtenberger et al. (1954 b) studied a number of tumours, including two bronchial carcinomata, one anaplastic and one adenocarcinoma. In both tumours, the basal modal value was equal to the diploid value of the normal tissue, and a group of cell nuclei with the double value was also observed. In the adenocarcinoma, one cell with an octaploid value was found per 30 cells, while none with values above the tetraploid range among 28 nuclei were found in the anaplastic carcinoma.

Boriani & Gandolfi (1961) investigated the DNA content in various bronchial tumours. The results were stated in arbitrary units and compared with the content of the nuclei of normal bronchial epithelium, viz. 10 18 A. U The figures reported were: prickle-cell carcinoma, 52.85–104 67 A. U., polymorphous epithelioma, 59.55–73 92 A. U., microcytoma,

(1960) found a considerable decrease in the content of DNA and RNA in the parts of the tumour which showed grossly visible signs of necrosis. Even when visible necrosis was absent in the central part of a tumour the amounts of both DNA and RNA were reduced. However this loss was not as great as in tumours with grossly visible necrosis. Early necrosis may be demonstrated by a decrease in the DNA content. After ichaemia of the liver for 60–90 minutes, an irreversible reduction in the content of DNA in the cell nuclei occurred (James 1968)

Sandritter (1965) studied the content and the distribution of DNA in two tumours and found the same values peripherally and centrally in the cords of tumour cells.

Nuclear RNA

The nuclei of cancer cells will often appear dark and hyperchromatic, the hyperchromasia being a reflection of the ability of the nuclei—depending on the pH level—to bind basic stains

The calculation of the nuclear RNA content of cancer cells is very difficult. Most investigations of the nuclear RNA content have been made by biochemical methods. It is, however difficult to isolate the nucleus and determine the RNA content without contaminating it with cytoplasmic RNA and without loss of RNA

In microphotometry this determination is extremely difficult, as it will require measurements of the nucleic acid content in the nuclei without and with RNase treatment. On account of the very great dispersion of the nucleic acid content in the nuclei of cancer cells, a study of this character will have to comprise an enormous number of cell nuclei.

In chemical determinations and ultraviolet microphotometry Leuchtenberger et al (1952 a, 1952 b) found a content of RNA in tumour-cell nuclei of 20–25 % of the total nucleic acid content, as compared with only 3–7 % in normal cell nuclei.

It appears from a study of Goldberg et al (1950) that the chemically determined nuclear RNA content constituted about 16 % of the total nucleic acid content of the tumour-cell nucleus. In spontaneous leukaemia in mice, Peterman & Schneider (1951) found that the nuclear RNA was increased 1 6 times, while the nuclear RNA content in transplanted leukaemia was increased 4 2 times, as compared with normal It appears that the nuclear RNA in normal lymphocyte nuclei constituted about 4 3 % of the total nucleic acid content of the nuclei.

DNA Content of Malignant Tumours

The DNA content of normal cell nuclei is very constant, and shows only a slight variation on account of aneuploidity Variations in the DNA content are mainly due to DNA synthesis in preparation for mitosis

The DNA content in tumour tissue is characterized by a higher average level and by a wide dispersion of the values. The distribution curve for the DNA values in tumour tissues shows an accumulation of cells around a basal modal value which tends to be localized around the somatic diploid value (Koller 1960) or within the hyperdiploid range However it may also be found within the hypodiploid or tetraploid range. In addition, another peak is often seen at about twice the modal value. This is largely due to cells which have synthetized DNA in preparation for mitosis There are many intermediate values, which are due either to cells synthetizing DNA prior to mitosis, or to cells with an aneuploid DNA content (Bader 1959 Bader et al 1960) It is also characteristic of malignant tumours that DNA values above twice the modal DNA value are nearly always found

Increased mitotic activity is not presumed to be a contributory factor in carcinogenesis (Bader et al 1960) even though intense mitotic activity is often found in malignant tumours (Bertalanffy & Lau 1962 b)

In certain tumours, the mitotic activity is lower than in the corresponding normal tissue

It may occasionally be difficult to investigate the chromosomes in solid tumours as mitoses may either be rare or technically too bad for proper assessment (Makino et al. 1959)

An essential disadvantage of microphotometric investigations of the DNA content is that small numerical and structural aberrations of the chromosomes cannot be recorded. In chromosome studies, the number and morphology of the chromosomes can be accurately assessed when the material is suitable for this purpose (Hsu 1961).

Chromosomes in Tumour Tissue

Since the end of the last century an aetiological relationship between chromosome aberrations and malignancy has been assumed. Winge was the first to emphasize that tumour tissue consists of a population of heterogeneous cells, and that the origin and growth of a tumour are a selective process among the cells in this population (Levan 1956).

Mitotic irregularities are often seen in malignant tumour tissue, and malignant cells often contain an abnormal number of chromosomes (Levan 1956, Ising & Levan 1957) Whether these chromosome abnormalities are of primary significance, or secondary to the special environment in which they exist, is still a subject of discussion (Koller 1960) although most investigators are inclined to believe that they are primary (Hamerton 1961)

Chromosome investigations on experimentally induced ascitic tumours with demonstration of stem lines have shown that there is a certain degree of order in the chromosome conditions in tumour tissue. In each tumour there is a population of cells occurring with great frequency which has a characteristic chromosome pattern, and in which the behaviour of the chromosomes is absolutely regular during mitosis. These tumour cells contain a fixed modal number of chromosomes and form the stem line of the tumour (Makino 1957).

Such a cell type with a fixed modal number of chromosomes was demonstrated by Ford et al. (1958) in a number of tumours. The chromosomes in the tumour tissue differed both in number and morphology from those of normal cells. In all the tumours studied there was a certain variation in the number of chromosomes around the modal value, as distinct from the very slight variation in normal cell nuclei.

Variations in the chromosome number are due partly to a doubling of the chromosomes, either on account of errors in separation at anaphase or on account of doubling by endomitosis, and partly to a reduction in the chromosome number because of irregular multipolar spindles (Hauschka & Levan 1951), and to a continuous fluctuation around the modal value on account of disturbances at anaphase. These disturbances, which give rise to formation of aneuploid cells, probably occur with an appreciable frequency in tumour tissue. By scanning of Feulgen-stained sections of tumour nuclei at anaphase and telophase, Barka (1959) found that significant differences in the DNA content in the daughter cells occurred in three different tumours with a frequency of 41.5 % 16.7 % and 18 7 %

Chromosome studies are performed on cells at metaphase. These cells with a modal number of chromosomes are able to divide and are responsible for the growth of the tumour On the other hand, there are probably many other cells in tumour tissue which cannot be studied in this way as many aneuploid cells, in all probability are unable to synthetize DNA and form metaphases (Makino 1957 Hauschka 1963) It is possible that such aneuploid cells are not viable (Makino 1957 Stich & Steele 1962, Hauschka 1963 Stich 1963 Richards et al. 1956). Some are perhaps able to produce metaphases, but unable to complete the division (Bader 1959 Stich & Steele 1962)

In relation to the diploid value, which is found in normal cells of the organism, the stem line in tumours is most often situated within the hyperdiploid range, but may assume all values from hypodiploid through diploid and hyperdiploid to tetraploid or even higher Most commonly there will be one stem line, but

74 09–66 12 A U The average DNA content of all the tumours was thus considerably higher than that of the nuclei of normal bronchial epithelium.

Sandritter & Fischer (1962) studied the DNA content in a number of tumours including two small-celled bronchial carcinomata. Both had two peaks on the distribution curve one at the basal modal value within the hypodiploid and *the triploid range, respectively* and the second peak at the double value. In both cases, it appears from the distribution curve that there were also cell nuclei with values above the highest peak.

Sandritter & Kleinhans (1964) studied the dry weight, DNA content and histone content in eight different tumours, including five bronchial carcinomata, one small-celled and four squamous-cell carcinomata. The modal value *for the small-celled* carcinoma was within the hypodiploid range for the squamous-cell carcinomata it was within the diploid range yet with one exception within the hyperdiploid range. No relation was found between the *measurements* (the *distribution* pattern of DNA and the position of the stem line) on the one hand and the clinical course and histological structure on the other

The DNA content was studied microphoto*metrically in three squamous-cell carcinomata* by Sandritter Seidel et al (1965) The distribution curves show DNA values from diploid to hypertetraploid values in two cases, while the third case has a modal value within the tetraploid range with values extending into and even beyond the octaploid range

In ultraviolet absorption measurements on bronchial carcinomata, Hührer & Kaffenberger (1962) studied the nucleic acid content in two small-celled carcinomata one squamous-cell carcinoma and one adenocarcinoma. A great dispersion of the nucleic acid content was *found in* all the tumours, from the modal value up to the hypertetraploid range In one of the small-celled bronchial carcinomata, the average value was *of the same order as in the* normal cell nuclei but it appears from the figure that the modal value for this tumour was within the hypodiploid range.

In a study of scrapings from three bronchial squamous-cell carcinomata, Sandritter Müller et al *(1958)* found that *91 %, 50 %* and 73 % of the cells had a nucleic acid content exceeding $1^1/_2 \times$ the diploid value, and in one small-celled bronchial carcinoma 61 % of the cells had a DNA content above that value.

DNA Content—Chromosomes

Good agreement between microphotometric determinations of the DNA content in stem lines and the number of chromosomes was found by Leuchtenberger et al (1954 b), Klein (1955) Richards et al (1956) and Sandritter Hilwig et al (1965)

DNA determinations have certain adva tages over chromosome studies. *They are i* dependent of the structure of the tumou Both solid tumours and suspensions of canc cells may be used in such studies. The fr quency of mitoses is of no significance *connection with the measuring technique* Th material need not be freshly prepared and is easier to make a random selection of the t mours to be studied As contrasted with chr mosome studies, DNA determinations do n *require cells capable of forming* metaphase this is an advantage, as we cannot be su that all tumour-cell nuclei can do so (Makir 1957 Ising & Levan 1957) By means of micr photometry it is possible to study cells at stag of mitosis other than metaphase (Richards al 1956)

Finally microphotometric determinations a *probably the only way in which precancero* cells can be studied in the early stages c cancer as chromosome studies do not allo differentiation of tumour cells from norm cells, which will also divide (Hsu 1961) Photo metric investigations can be performed on his tological sections, which permits a definite mor phological identification of the cells in whicl *the nuclei* are to be studied (Emson & Kirl 1967)

in the tetraploid strain, these cells were less virulent, less invasive and required longer time to kill the animals than the more virulent diploid lymphomata (Hauschka et al. 1956)

Polyploid cells which developed by endomitosis without spindle formation were observed by Geitler (1939) in an insect. A few human organs will under normal conditions contain polyploid cell nuclei, viz. the liver and bone marrow (megakaryocytes) (Hauschka 1963). Furthermore, polyploid cells have been described in the pancreas and in testes in mice, and in various other organs in insects (Swift 1950)

In certain circumstances with hormonal overstimulation, polyploid cell nuclei may develop in non-neoplastic tissue. This applies, for example, to the endometrium (Wagner et al. 1968), the thyroid gland (Beierwaltes et al. 1966) the liver (Swartz 1956) the liver and pancreas (Leuchtenberger et al. 1954 a) Polyploid nuclei have also been observed in the urinary bladder (Walker 1958) and in muscle cells of hypertrophic human hearts (Kompmann et al. 1966)

According to the literature and my own investigations, polyploid nuclei are absent in normal bronchial epithelium.

The formation of polyploid nuclei may occur after the synthesis of DNA by an inhibition of mitosis, either by an inhibition of the duplication of the chromosomes or of their movements (Gelfant 1963)

Polyploidity is not necessarily a multiple of the euploid chromosome number as polyploidity represents all disturbances in the relation between the reproduction and the distribution of the chromosomes in the daughter cells (Levan & Hauschka 1953)

C-mitotic duplication, which resembles colchicine-induced mitotic inhibition, is due to failure in the kinetic apparatus.

Endomitosis involves a division of the contracted chromosomes inside the intact nuclear membrane, leading to endometaphase and back again to the despiralized stage. Orientation of the spindle is lacking, and the chromosomes are evenly distributed inside the nuclear membrane without formation of an equatorial plate of chromosomes at metaphase. The chromosomes are not arranged in pairs, and the daughter chromatids are unable to move from each other before the next mitosis.

In endoreduplication, a doubling of the chromosomes occurs in the despiralized stage inside the nuclear membrane without any mitosis-like manifestation. The chromosomes do not move from each other but persist as diplo-chromosomes until the next mitosis (Levan & Hauschka 1953) The high polyploid DNA content at interphase may reflect repeated endomitoses (Hauschka 1963)

Another mode of formation of polyploid nuclei was demonstrated by Robberts & Cole (1964) In ascitic tumour cells, polyploid cells could be formed from uninuclear cells with lower ploidity through an intermediate stage of binuclear cells. The binuclear cell was the result of lacking cytoplasmic cleavage in connection with the cell division. When the binuclear cell approached the next division, the chromosomes in both nuclei were united, and two uninuclear cells with a higher ploidity was found.

From numerous investigations of the DNA content and chromosomes in tumour tissue it appears, in the descriptions of cell distributions and in the histograms, that polyploid cell nuclei are found in many cases. However only a few indications of the frequency of these polyploid cell nuclei are available. Ishihara et al. (1963) found polyploid cell nuclei in pleural and ascitic fluids from cancer patients with a frequency of 10–40 % of the cell number At kin et al. (1959) studied the DNA content in a number of uterine carcinomata and found in a few tumours giant nuclei with values from 4 to 16 times the modal value. These cells occurred fairly often, but the frequency was not definitely stated. Koller (1956) mentioned that the frequency of polyploid, polynuclear degenerating and abnormally dividing cells in solid tumours is higher in differentiated than in anaplastic malignant tumours.

cases with two stem lines have also been reported.

When two stem lines exist they are usually situated very close to each other with a difference of only a few chromosomes Several of these tumours with two stem lines have been given radiotherapy or chemotherapy In two cases, modal chromosome numbers at a great distance from each other have been observed In both cases, the tumour had been subjected to radiotherapy and in both the highest peak was equal to twice the lower value (Hansen Melander et al 1956 Makino et al 1959 Ishihara et al 1963 Makino et al 1964 Atkin 1967) The cell group with the chromosome number at the double value was presumably produced from that with the low modal value. It is known that radiation therapy can produce doubling of the chromosome number by endomitosis and endoreduplication (Caspersson et al 1958 Richards & Atkin 1959 Bell & Baker 1962) and give rise to variations in the number and morphology of the chromosomes (Conger 1956 Upton 1963)

Polyploid Cell Nuclei

By polyploid cells is understood cells containing more than the diploid number of chromosomes (DeRobertis et al 1965) In analogy with this and with due allowance for premitotic DNA synthesis, polyploid cell nuclei may in microphotometry be defined as nuclei with a DNA content which is higher than twice the basal modal value.

As already mentioned in a population of cancer cells, there will be a certain variation in the number of chromosomes around the modal number of chromosomes of the tumour concerned This modal number of chromosomes is relatively constant in each tumour but genetic instability is a characteristic feature in cancer tissue and changes in the chromosomes may occur spontaneously without any explainable reason or during alterations in the growth conditions (Oberling & Bernhard 1961)

Levan & Biesele (1958) studied the cytological changes in embryonic tissue culture They demonstrated a gradually increasing number of polyploid cells at the expense of diploid cells. First a shift from diploidity to tetraploidity occurred, followed by a wider dispersion of the values above and below the tetraploid value and, finally by the formation of a heteroploid stem line with a chromosome number between the diploid and the tetraploid value A similar transformation was reported by Hsu & Klatt (1959)

In tissue cultures, substances may be formed which on accumulation affect the cells and may act as weak mitotic poisons. The rapid shift from diploid to tetraploid indicates either that tetraploid cells are more resistant to noxious substances than the diploid ones, resulting in a higher survival rate of the tetraploid cells, or that the diploid cells under the influence of a mitotic inhibitor in the medium are unable to undergo cytokinesis and therefore will become tetraploid (Hsu 1961)

Provided that the vital processes are preserved, a tetraploid cell is from a theoretical point of view only half as efficient as a diploid cell in mitosis. In order to divide once the former must synthetize twice as much material as the latter (Hsu 1961) This was shown experimentally by Hauschka et al (1957) in two ascitic tumours of identical genetic constitution. The hypertetraploid stem represented the duplication of the hyperdiploid stem and was isolated by selection from the hyperdiploid stem After transplantation, the number of cells was twice as large in the hyperdiploid as in the hypertetraploid stem after the first initial stage of growth, while the total tumour mass was equal in both tumours. The average generation time and mitotic time of the cells were twice as long in the hypertetraploid as in the hyperdiploid strain

In three mouse ascitic tumours isolated from the same highly virulent ascitic lymphosarcoma strain, the modal value of two of the tumours were near-diploid while one was tetraploid. In spite of a slight iso-antigenic effect of the cells

In the tetraploid strain, these cells were less virulent, less invasive and required longer time to kill the animals than the more virulent diploid lymphomata (Hauschka et al. 1956)

Polyploid cells which developed by endomitosis without spindle formation were observed by Geitler (1939) in an insect. A few human organs will under normal conditions contain polyploid cell nuclei, viz. the liver and bone marrow (megakaryocytes) (Hauschka 1963) Furthermore, polyploid cells have been described in the pancreas and in testes in mice, and in various other organs in insects (Swift 1950).

In certain circumstances with hormonal overstimulation, polyploid cell nuclei may develop in non-neoplastic tissue. This applies, for example, to the endometrium (Wagner et al. 1968), the thyroid gland (Beierwaltes et al. 1966) the liver (Swartz 1956) the liver and pancreas (Leuchtenberger et al. 1954 a) Polyploid nuclei have also been observed in the urinary bladder (Walker 1958) and in muscle cells of hypertrophic human hearts (Kompmann et al. 1966)

According to the literature and my own investigations, polyploid nuclei are absent in normal bronchial epithelium.

The formation of polyploid nuclei may occur after the synthesis of DNA by an inhibition of mitosis, either by an inhibition of the duplication of the chromosomes or of their movements (Gelfant 1963)

Polyploidity is not necessarily a multiple of the euploid chromosome number as polyploidity represents all disturbances in the relation between the reproduction and the distribution of the chromosomes in the daughter cells (Levan & Hauschka 1953)

C-mitotic duplication, which resembles colchicine-induced mitotic inhibition, is due to failure in the kinetic apparatus.

Endomitosis involves a division of the contracted chromosomes inside the intact nuclear membrane, leading to endometaphase and back again to the despiralized stage. Orientation of the spindle is lacking, and the chromosomes are evenly distributed inside the nuclear membrane without formation of an equatorial plate of chromosomes at metaphase. The chromosomes are not arranged in pairs, and the daughter chromatids are unable to move from each other before the next mitosis.

In endoreduplication, a doubling of the chromosomes occurs in the despiralized stage inside the nuclear membrane without any mitosis-like manifestation. The chromosomes do not move from each other but persist as diplo-chromosomes until the next mitosis (Levan & Hauschka 1953) The high polyploid DNA content at interphase may reflect repeated endomitoses (Hauschka 1963)

Another mode of formation of polyploid nuclei was demonstrated by Robberts & Cole (1964) In ascitic tumour cells, polyploid cells could be formed from uninuclear cells with lower ploidity through an *intermediate stage* of binuclear cells. The binuclear cell was the result of lacking cytoplasmic cleavage in connection with the cell division. When the binuclear cell approached the next division, the chromosomes in both nuclei were united, and two uninuclear cells with a higher ploidity was found.

From numerous investigations of the DNA content and chromosomes in tumour tissue it appears, in the descriptions of cell distributions and in the histograms, that polyploid cell nuclei are found in many cases. However only a few indications of the frequency of these polyploid cell nuclei are available. Ishihara et al. (1963) found polyploid cell nuclei in pleural and ascitic fluids from cancer patients with a frequency of 10–40 % of the cell number At kin et al. (1959) studied the DNA content in a number of uterine carcinomata and found in a few tumours giant nuclei with values from 4 to 16 times the modal value. These cells occurred fairly often, but the frequency was not definitely stated. Koller (1956) mentioned that the frequency of polyploid, polynuclear degenerating and abnormally dividing cells in solid tumours is higher in differentiated than in anaplastic malignant tumours.

cases with two stem lines have also been reported.

When two stem lines exist, they are usually situated very close to each other with a difference of only a few chromosomes. Several of these tumours with two stem lines have been given radiotherapy or chemotherapy In two cases, modal chromosome numbers at a great distance from each other have been observed In both cases the tumour had been subjected to radiotherapy and in both the highest peak was equal to twice the lower value (Hansen-Melander et al 1956 Makino et al 1959 Ishihara et al 1963 Makino et al 1964 Atkin 1967) The cell group with the chromosome number at the double value was presumably produced from that with the low modal value. It is known that radiation therapy can produce doubling of the chromosome number by endomitosis and endoreduplication (Caspersson et al 1958 Richards & Atkin 1959 Bell & Baker 1962) and give rise to variations in the number and morphology of the chromosomes (Conger 1956 Upton 1963)

Polyploid Cell Nuclei

By polyploid cells is understood cells containing more than the diploid number of chromosomes (DeRobertis et al 1965) In analogy with this and with due allowance for premitotic DNA synthesis, polyploid cell nuclei may in microphotometry be defined as nuclei with a DNA content which is higher than twice the basal modal value

As already mentioned, in a population of cancer cells, there will be a certain variation in the number of chromosomes around the modal number of chromosomes of the tumour concerned This modal number of chromosomes is relatively constant in each tumour but genetic instability is a characteristic feature in cancer tissue and changes in the chromosomes may occur spontaneously without any explainable reason or during alterations in the growth conditions (Oberling & Bernhard 1961)

Levan & Biesele (1958) studied the cytological changes in embryonic tissue culture. They demonstrated a gradually increasing number of polyploid cells at the expense of diploid cells. First a shift from diploidity to tetraploidity occurred, followed by a wider dispersion of the values above and below the tetraploid value and finally by the formation of a heteroploid stem line with a chromosome number between the diploid and the tetraploid value A similar transformation was reported by Hsu & Klatt (1959)

In tissue cultures substances may be formed which, on accumulation, affect the cells and may act as weak mitotic poisons. The rapid shift from diploid to tetraploid indicates either that tetraploid cells are more resistant to noxious substances than the diploid ones, resulting in a higher survival rate of the tetraploid cells, or that the diploid cells under the influence of a mitotic inhibitor in the medium are unable to undergo cytokinesis and therefore will become tetraploid (Hsu 1961)

Provided that the vital processes are preserved, a tetraploid cell is from a theoretical point of view only half as efficient as a diploid cell in mitosis. In order to divide once the former must synthetize twice as much material as the latter (Hsu 1961) This was shown experimentally by Hauschka et al (1957) in two ascitic tumours of identical genetic constitution. The hypertetraploid stem represented the duplication of the hyperdiploid stem and was isolated by selection from the hyperdiploid stem After transplantation, the number of cells was twice as large in the hyperdiploid as in the hypertetraploid stem after the first initial stage of growth while the total tumour mass was equal in both tumours. The average generation time and mitotic time of the cells were twice as long in the hypertetraploid as in the hyperdiploid strain.

In three mouse ascitic tumours isolated from the same highly virulent ascitic lymphosarcoma strain the modal value of two of the tumours were near-diploid while one was tetraploid. In spite of a slight iso-antigenic effect of the cells

modal DNA value, the clinical stage, the age of the patient or the survival time, a significantly better prognosis was found in cervical carcinomata with modal values within the tetraploid range than in tumours with modal values within the diploid range (Atkin et al. 1962, Atkin 1964 a) The incidence of lymph-node metastases was significantly higher in the diploid class. The ploidity and the histological degree of differentiation were correlated in such a manner that most undifferentiated tumours had diploid stem lines, while the more differentiated tumours had predominantly tetraploid stem lines. The relationship between ploidity and prognosis was, however independent of that between ploidity and histological degree of malignancy

In chromosome studies of seven adenocarcinomata of the colon, Enterline et al. (1967) found that aggressive, poorly differentiated carcinomata had the lowest, near-diploid modal values, while the cells in the other carcinomata usually had higher numbers of chromosomes.

In 135 carcinomata of the prostate, Jacobsen (1968) found no correlation between the average nucleic acid content of the nuclei and the duration of symptoms to death or survival time after operation.

Studies of the chromosomes in various tumours failed to reveal a correlation between the degree of malignancy and that of structural changes in the chromosomes. Nor was any correlation observed between ploidity and the histological degree of differentiation of the tumour (Makino et al. 1959 Ishihara et al. 1963 Makino et al. 1964)

DNA Content of Metastases

In the literature, only a few studies of the DNA content of metastases are on record.

Leuchtenberger et al. (1954 b) reported that the dispersion of DNA values in metastases is wider than in the primary tumour

In a study of the DNA content in a case of malignant adenomatosis of the lung with metastases to the lymph nodes, Seidel & Sandritter (1963) found that the average DNA content in the metastatic tumour cells was only slightly higher than in the primary tumour The dispersion of the DNA content was also slightly higher The distribution pattern of the DNA values in the primary tumour was, however similar to that in the metastasis.

The mitotic frequency in the metastatic tumour cells corresponded to that in the tumour cells which proliferated in ascites. The cells which primarily participate in the formation of the metastatic tumour are probably stem cells, just as in the primary tumour (Makino 1957)

Chromosome studies on a metastasis from a carcinoma of the uterine cervix showed conditions similar to those observed in the primary tumour (Wakonig-Vaartaja et al. 1967).

By means of microphotometric measurements, Chu et al. (1961) determined the DNA content in five spontaneous and 11 metastatic mouse tumours. All metastatic tumours had a higher ploidity than the primary tumour

In a study of the DNA content in metastasizing primary bronchogenic carcinoma, Stich & Steele (1962) found a higher modal value in the primary tumour than in the metastasis.

Cytoplasmic RNA

In rapidly growing tissue, a high concentration of RNA is found in the cytoplasm. This applies, for example, to embryonic tissue and glands, in which intense protein synthesis occurs. RNA is a prerequisite for the production of protein in the cytoplasm, both during growth and in functional activity (Caspersson 1947)

In the carcinoma, type-A cells are characterized by a high concentration of protein and RNA in the cytoplasm, a large content of DNA in the nucleus, and well-developed nucleoli. This indicates a brisk activity of the protein-producing system in the cytoplasm and nucleus. Type-A cells are thus engaged in extremely intense growth and division, and the growth rate may be evaluated on the basis of the protein-producing system. The cytoplasm of type-B cells contains only a small amount

Chromosomes in Human Malignant Tumours

The chromosome constitution of 20 pleural and ascitic fluids from cancer patients was studied by Ishihara et al (1963) One of the tumours was a lung cancer (histological type not stated) The modal chromosome number was 101–102, i. e. within the hypertetraploid range. The wide dispersion of the chromosome number was a characteristic feature. There was no correlation between the degree of malignancy the modal number of chromosomes and structural changes in the chromosomes

In a case of anaplastic bronchogenic adenocarcinoma and one of gastric carcinoma the modal numbers of chromosomes were 75 and 82 respectively with a wide variation around the modal chromosome numbers. In both cases there was also a large number of cells with a considerably larger number of chromosomes, viz. 150 and 220 respectively These giant cells with the large number of chromosomes were presumed to have developed by endomitosis, because endometaphases occurred with a high frequency in both tumours (Ising & Levan 1957)

Makino et al. (1959) studied the chromosome constitution in 30 human tumours, including three squamous-cell bronchial carcinomata, which all had near-diploid stem lines. Incidentally the stem line was in most cases situated within the near-diploid or the hyper diploid range In some cases the stem line was near triploid and in a few it was hypotetraploid. A similar distribution of the stem lines was found in series of human tumours investigated by Makino et al (1964) but no correlation was demonstrated between the position of the stem line and the origin of the tumour or between the chromosome constitution and the histopathological structure of the tumour

In a series of 36 tumours of the female genital system, Wakonig Vaartaja et al (1967) found that the stem line might assume all values from hypodiploid to hypotetraploid In each type of tumour the number of cells with chromosome numbers within the near-diploid the near triploid and the near tetraploid range and within the range above this was stated.

In most human tumours, one particularly frequent cell type forming the stem cell of the tumour can be determined Most frequently there will be one stem line, but occasionally there may be two or even more. The stem lines from human tumours are usually localized within the near-diploid or the hyperdiploid range, but may vary from the hypodiploid to the tetraploid range. No relationship between the clinical course and the histopathological structure and chromosome pattern has been reported

DNA Content and Prognosis

Studying the average content of DNA and RNA in 14 squamous-cell carcinomata (including three bronchogenic) and in five small-celled carcinomata (all from the bronchi) Sandritter (1952 a) found that the cell division rate in normal cells and in tumour cells did not depend on the content of DNA and RNA It was concluded that the degree of malignancy in a tumour does not depend on the content of nucleic acids (Sandritter 1952 a 1952 b)

In a study of the DNA content in normal epithelium, in adenomatous polyps and in adenocarcinomata from the human colon, Stich et al (1960) failed to demonstrate any correlation between the degree of malignancy as assessed by histological examination and a certain aneuploid DNA content of the tumour cells.

In a number of tumours, including one small-celled bronchial carcinoma and four squamous cell bronchial carcinomata no correlation was demonstrated between the content and distribution pattern of DNA the position of the stem line and the clinical course and the histological structure of the tumour (Sandritter & Kleinhans 1964)

While Atkin et al (1959) in a study of the DNA content in carcinomata of the uterine cervix and corpus were unable to demonstrate a significant correlation between the basal

modal DNA value, the clinical stage, the age of the patient or the survival time, a significantly better prognosis was found in cervical carcinomata with modal values within the tetraploid range than in tumours with modal values within the diploid range (Atkin et al. 1962, Atkin 1964 a). The incidence of lymph-node metastases was significantly higher in the diploid class. The ploidity and the histological degree of differentiation were correlated in such a manner that most undifferentiated tumours had diploid stem lines, while the more differentiated tumours had predominantly tetraploid stem lines. The relationship between ploidity and prognosis was, however independent of that between ploidity and histological degree of malignancy

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Cytoplasmic RNA

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of protein and nearly no RNA They have large nuclei with only a small amount of nu cleic acids and very large nucleoli with a high *content of RNA Type B cells show a less in* tense growth activity with only slight protein synthesis (Caspersson & Santesson 1942)

The RNA content in tumour tissue was studied by Stowell (1946) The figures reported apply to the entire cell, i e cytoplasm plus nucleus. In many cases, there was no difference between the RNA content in tumour tissue and the corresponding normal tissue, but, in other cases, the tumour tissue revealed either a higher or a smaller RNA content than the normal tissue.

Cytophotometric investigations of gallo-cyanin-chromalum-stained tissue performed by Grundmann et al. (1960) showed that the epi thelial cytoplasm in the cervix of the uterus in precancerous stages contained more RNA than the normal epithelium

In a study of the RNA content of bronchogenic carcinomata, including three squamous-cell and five small-celled ones, Sandritter (1952 a) found that the RNA content of the small-celled bronchial carcinoma (stated for the entire cell i. e. nucleus plus cytoplasm) was considerably lower than that of the cells of the squamous-cell carcinoma. He mentioned that the small-celled bronchial carcinomata range among the most rapidly growing tu mours, and that they have a great propensity to metastasize. The high degree of malignancy with the high cell-division rate will provide little time for the construction of the protein forming system and thus Caspersson s theory as to cytoplasmic RNA was not confirmed as far as bronchogenic carcinomata were con cerned

Sandritter Müller et al (1958) studied the RNA concentration in sputum cells It was found to be considerably higher in the cytoplasm of squamous-cell bronchial carcinomata than in the ciliated cylindrical cells from the bronchial epithelium, but lower than in the cytoplasm of normal squamous cells from the oral mucosa.

Personal Investigations

The material studied consisted of biopsy specimens from 34 bronchogenic carcinomata, viz. squamous-cell carcinoma, 13 adenocarcinoma, 8 (two from the same patient, viz. B 11 and B 12) and small-celled anaplastic carcinoma, 13 (two from the same patient viz. N 5 and N 6)

The biopsy specimens are comparable as far as the localizations of the tumours are concerned all the biopsy specimens were removed by bronchoscopy and are thus from central tumours (see page 70)

None of the patients had been given radia tion therapy or been treated with cancer chemotherapeutic agents when the biopsy was performed

Nuclear Measurements

The nuclear measurements were all performed on RNase treated sections.

In each section 30–40 regular non-sec tioned nuclei selected at random, were subjected to measurements The values for the DNA content of the first 30 nuclei were used in the calculation of the average DNA content. The additional nuclei were measured in order to obtain a better evaluation of the position of the modal value.

Characteristic features of the DNA content in the bronchial carcinomata are as in other carcinomata the wide dispersion of the values and the high average content of DNA in the nuclei

The histograms in figure 38 show examples of the distribution of the DNA content in 30 nuclei from a squamous-cell carcinoma an adenocarcinoma and a small-celled anaplastic carcinoma.

After the calculation of the DNA content of the carcinoma nuclei the DNA values for each section were plotted in a histogram In this way it is possible with a reasonable de gree of certainty to assess the position of the modal value after measurements on 30–40 tu mour-cell nuclei (Atkin et al 1966)

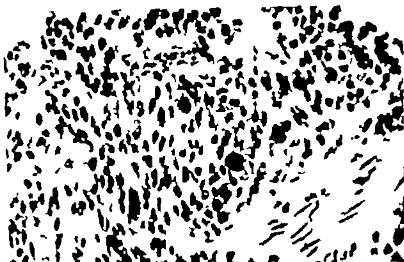


Fig. 35.—Squamous-cell carcinoma (gallocyanin-chromalum, × 350).

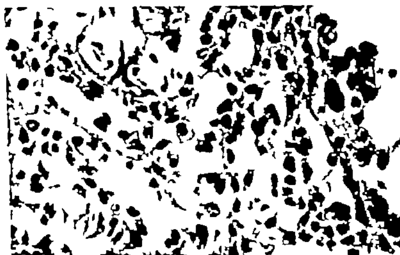


Fig. 36.—Adenocarcinoma (gallocyanin-chromalum, × 350)

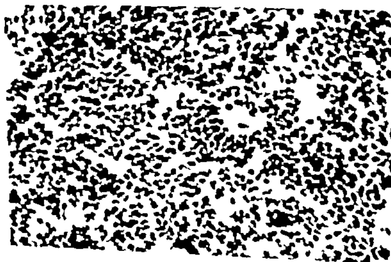


Fig. 37.—Small-celled anaplastic carcinoma (gallocyanin-chromalum, × 350)

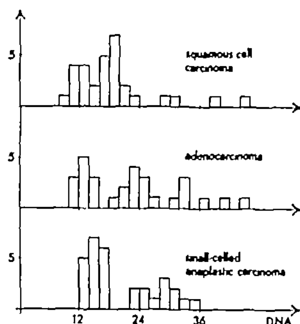


Fig. 38.—Distribution patterns of DNA content in squamous-cell carcinoma (section No E 26) adenocarcinoma (section No. B 6) and small-celled anaplastic carcinoma (section No. N 9)

Although no measurements were performed on sections showing necrosis, there may nevertheless be *measurable signs of this process* (see page 72) In order to assess this and to compare the DNA values for the various sections, the DNA content in each section was measured in cells with a normal *diploid* DNA content. Whenever possible the DNA content was therefore measured in normal epithelial nuclei when no epithelial tissue was available in the section it was measured in lymphocytes, which were nearly always present in the section. As appears from tables 35 36 and 37 there is some dispersion of the diploid values in the sections. This may be due partly to non visible, but measurable necrosis in the tissue and partly to technical or measuring errors in the sections.

In nearly all cases, the average DNA con-

Table 35 *Average DNA content and modal value in squamous-cell carcinomata*

Section No	Average DNA content A. U	Corrected DNA content A. U	Modal value A. U	Diploid value A. U	Modal/diploid value
F 1	25.5	28.1	14.5	10.9	1.33
E 6	13.1	16.7	10.5	9.4	1.12
E 9	11.7	16.3	7.5	8.6	0.87
E 10	12.7	15.7	9.5	9.7	0.98
E 11	19.6	23.5	13.5	10.0	1.35
E 17	23.7	22.9	15.5	12.4	1.25
E 24	29.8	28.4	15.5	12.6	1.[illegible]3
F 26	18.7	18.2	11.5	12.3	0.93
E 8	26.5	28.4	16.5	11.[illegible]	1.47
E 29	[illegible]5.6	25.8	16.5	11.9	1.39
F 32	23.2	[illegible]5.1	15.5	11.1	1.40
E 33	26.4	[illegible]8.5	16.5	11.1	1.49
E 34	24.7	23.0	16.0	1[illegible].9	1.24
Average	[illegible]1.6	[illegible]3.1	13.8	11.1	

Table 36 *Average DNA content and modal value in adenocarcinomata*

Section No	Average DNA content A. U	Corrected DNA content A U	Modal value A. U	Diploid value A. U	Modal/diploid value
B 5	19.1	15.7	13.5	14.6	0.92
B 6	22.7	21.3	13.0	12.8	1.02
B 9	[illegible]9.7	29.5	15.0	1[illegible].1	1.24
B 11	[illegible]3.5	22.6	15.0	1[illegible].5	1.20
B 12	[illegible]6	[illegible]8.4	13.5	10.8	1.[illegible]5
B 13	[illegible]0.5	20.0	13.5	1[illegible].3	1.10
B 16	[illegible]6.6	29.6	11.5	10.8	1.[illegible]5
B 18	[illegible]6.0	6[illegible]	15.8	11.9	1.33
Average	4[illegible]	4[illegible]	14.1	1[illegible]	

Table 37 *Average DNA content and modal value in small-celled anaplastic carcinomata.*

Section No.	Average DNA content A. U.	Corrected DNA content A. U	Modal value A. U	Diploid value A. U	Modal diploid value
N 1	16.0	19.4	15.0	9.9	1.51
N 4	13.2	15.2	10.0	10.4	0.96
N 5	11.2	13.0	10.0	10.3	0.97
N 6	12.6	–	11.5	–	–
N 7	10.6	15.9	7.5	8.0	0.94
N 8	15.9	–	10.0	–	–
N 9	20.4	28.1	15.0	8.7	1.72
N 10	10.6	17.9	7.5	7.1	1.06
N 11	19.8	–	14.5	–	–
N 12	19.4	20.8	15.5	11.2	1.38
N 21	24.4	23.1	17.5	12.7	1.38
N 23	25.5	4.9	16.5	12.3	1.34
N 27	18.4	17.0	1[illegible]0	13.0	0.92
Average	16.8	19.5	12.5	10.4	

tent in the tumour sections is considerably higher than that in the normal tissue. When corrected for measurable necroses and for technical and measuring errors by relating the average values of the tumours to a diploid value, fixed arbitrarily at 12.0 A. U., the values become comparable (listed as corrected DNA content in the tables). With the exception of a couple of the anaplastic carcinomata, the average DNA content was in all other cases considerably higher than the diploid value in the cell nuclei of the normal epithelium or in the lymphocytes. The average DNA content in all sections was higher in the squamous-cell and adenocarcinomata than in the small-celled anaplastic carcinoma.

The position of the modal value is stated in relation to diploid and near-diploid, triploid and near-triploid, tetraploid and near-tetraploid values. The limits were fixed so that the relation between the modal value and the diploid value in these ranges was 0.75–1.25, 1.25–1.75 and 1.75–2.25 respectively (Wakonig-Vaartaja et al. 1967) (table 38)

The distribution and position of the modal value in the various types of carcinoma appear from table 38. It is seen that there is no difference in the position of the modal value in the various groups, as about half of the tumours had a modal value within the diploid or near diploid range, while in the other half it was within the triploid or near-triploid range.

The modal value, as fixed by DNA determinations, will most often be an expression of one stem line, but as already mentioned, there may be more than one stem line. If so, they are most frequently situated very close to each other and will not to any considerable degree make the determination of the modal value by DNA measurements difficult. As none of the

Table 38 *Modal values of bronchogenic carcinomata.*

	Diploid Near-diploid	Triploid Near-triploid	Tetraploid Near-tetraploid	Total
Squamous-cell carcinoma	6	7	0	13
Adenocarcinoma	5	3	0	8
Small-celled anaplastic carcinoma	5	5	0	10
Total	16	15	0	31

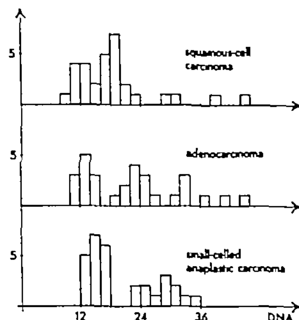


Fig. 38.—Distribution patterns of DNA content in squamous-cell carcinoma (section No. E ?6), adenocarcinoma (section No. B 6) and small-celled anaplastic carcinoma (section No. N 9)

Although no measurements were performed on sections showing necrosis, there may nevertheless be measurable signs of this process (see page 72) In order to assess this and to compare the DNA values for the various sections, the DNA content in each section was measured in cells with a normal diploid DNA content. Whenever possible, the DNA content was therefore measured in normal epithelial nuclei when no epithelial tissue was available in the section it was measured in lymphocytes, which were nearly always present in the section. As appears from tables 35 36 and 37 there is some dispersion of the diploid values in the sections. This may be due partly to non-visible but measurable necrosis in the tissue and partly to technical or measuring errors in the sections.

In nearly all cases, the average DNA con-

Table 35 *Average DNA content and modal value in squamous-cell carcinomata.*

Section No.	Average DNA content A. U	Corrected DNA content A. U	Modal value A. U	Diploid value A. U	Modal diploid value
E 1	25.5	28 1	14.5	10.9	1.33
E 6	13 1	16 7	10.5	9 4	1 1
E 9	11 7	16.3	7.5	8.6	0.87
E 10	12.7	15 7	9.5	9 7	0 98
E 11	19 6	23.5	13.5	10 0	1.35
E 17	_3 7	2_9	15.5	12.4	1_5
E 4	29 8	8 4	15.5	1_6	1.23
E 6	18 7	18._	11.5	12.3	0 93
E 28	26.5	8.4	16.5	11._	1 47
E 29	_5.6	_5 8	16.5	11.9	1.39
E 3	_3.2	25 1	15.5	11 1	1 40
E 33	26 4	28.5	16.5	11 1	1 49
E 34	24 7	23 0	16 0	1_9	1_4
Average	1.6	3 1	13 8	11 1	

Table 36 *Average DNA content and modal value in adenocarcinomata*

Section No	Average DNA content A. U	Corrected DNA content A U	Modal value A. U	Diploid value A. U	Modal diploid value
B 5	19 1	15 7	13.5	14 6	0 9
B 6	2_7	_1.3	13 0	1 8	1 0?
B 9	?9 7	29.5	15 0	1 1	1_4
B 11	3.5	__6	15 0	1_5	1 0
B 1	25 6	28 4	13.5	10 8	1_5
B 13	0.5	20 0	13.5	1_3	1 10
B 16	6.6	_9.6	13.5	10 8	1_5
B 18	6.0	6_	15.8	11 9	1 33
Average	4_	_4.2	14 1	1_	

Table 37 *Average DNA content and modal value in small-celled anaplastic carcinomata.*

Section No	Average DNA content A. U	Corrected DNA content A. U	Modal value A. U	Diploid value A. U	Modal/diploid value
N 1	16.0	19.4	15.0	9.9	1.51
N 4	13.2	15.2	10.0	10.4	0.96
N 5	11.2	13.0	10.0	10.3	0.97
N 6	12.6	–	11.5	–	–
N 7	10.6	15.9	7.5	8.0	0.94
N 8	15.9	–	10.0	–	–
N 9	20.4	28.1	15.0	8.7	1.72
N 10	10.6	17.9	7.5	7.1	1.06
N 11	19.8	–	14.5	–	–
N 12	19.4	20.8	15.5	11.2	1.38
N 21	24.4	23.1	17.5	12.7	1.38
N 23	25.5	24.9	16.5	12.3	1.34
N 27	18.4	17.0	12.0	13.0	0.92
Average	16.8	19.5	12.5	10.4	

tent in the tumour sections is considerably higher than that in the normal tissue. When corrected for measurable necroses and for technical and measuring errors by relating the *average values of the tumours to a diploid* value, fixed arbitrarily at 12.0 A. U the values become comparable (listed as corrected DNA content in the tables). With the exception of a couple of the anaplastic carcinomata, the average DNA content was in all other cases considerably higher than the diploid value in the cell nuclei of the normal epithelium or in the lymphocytes. The average DNA content in all sections was higher in the squamous-cell and adenocarcinomata than in the small-celled anaplastic carcinoma.

The position of the modal value is stated in relation to diploid and near-diploid, triploid and *near-triploid*, tetraploid and *near-tetraploid* values. The limits were fixed so that the relation between the modal value and the diploid value in these ranges was 0.75–1.25 1.25–1.75 and 1.75–2.25 respectively (Wakonig-Vaartaja et al. 1967) (table 38)

The distribution and position of the modal value in the various types of carcinoma appear from table 38 It is seen that there is no difference in the position of the modal value in the various groups, as about half of the tumours had a modal value within the diploid or near diploid range, while in the other half it was within the triploid or near-triploid range.

The modal value, as fixed by DNA determinations, will most often be an expression of one stem line but as already mentioned, there may be more than one stem line. If so, they are most frequently situated very close to each other and will not to any considerable degree make the determination of the modal value by DNA measurements difficult. As none of the

Table 38. *Modal values of bronchogenic carcinomata.*

	Diploid Near-diploid	Triploid Near-triploid	Tetraploid Near-tetraploid	Total
Squamous-cell carcinoma	6	7	0	13
Adenocarcinoma	5	3	0	8
Small-celled anaplastic carcinoma	5	5	0	10
Total	16	15	0	31

Table 39 *Distribution of the nuclear DNA content (x) in relation to the modal value (M) in squamous-cell carcinomata*

Section No	No. of nuclei x < 1¼ M	No. of nuclei 1¼ M < x < 2½ M	No of nuclei 2½ M < x
E 1	11	15	4
E 6	18	1[illegible]	0
E 9	14	13	3
E 10	16	14	0
E 11	15	13	2
E 17	13	13	4
E 24	12	11	7
E 26	10	16	4
E 28	15	13	2
E 29	11	15	4
E 32	11	17	2
E 33	11	18	1
E 34	14	13	3
Total	171	183	36
Average	43 8 %	46.9 %	9.2 %

patients had received radiotherapy there is no reason to assume the presence of stem lines at a long distance from each other (see page 76)

As the average DNA content was higher in the squamous-cell and adenocarcinomata than in the small-celled anaplastic carcinoma, it may be of interest to analyse the distribution of the DNA values in the various types of carcinoma.

The distribution of the DNA content in the tumour cell nuclei was determined The number of cell nuclei was stated around the modal value (up to the modal value + 2½ × the standard deviation with a coefficient of variation of about 10 % this is equal to the modal value + 2½ × the modal value 10 = 1¼ × the modal value) in the range from here up to twice the modal value (up to twice the modal value + 2½ × the standard deviation The standard deviation of the cells with a DNA content of twice the modal value was assumed to be twice the standard deviation of the cells with the modal value, i. e. up to 2 × the modal value + 2½ × 2 × the modal value 10 = 2½ × the modal value) and finally above the value (polyploid cell nuclei) (tables 39 40 41)

Table 40 *Distribution of the nuclear DNA content (x) in relation to the modal value (M) in adenocarcinomata.*

Section No	No. of nuclei x < 1 / M	No of nuclei 1 / M < x < 2 / M	No of nuclei 2 M < x
B 5	1	18	0
B 6	11	14	5
B 9	5	17	8
B 11	14	13	3
B 12	11	15	4
B 13	14	12	4
B 16	9	17	4
B 18	15	9	6
Total	91	115	34
Average	37 9 %	47 9 %	14.[illegible] %

Table 41. *Distribution of the nuclear DNA content (x) in relation to the modal value (M) in small-celled anaplastic carcinomata*

Section No.	No. of nuclei < 1½ M	No. of nuclei 1½ M < x < 2½ M	No. of nuclei 2½ M < x
N 1	24	6	0
N 4	16	14	0
N 5	24	6	0
N 6	23	6	1
N 7	12	17	1
N 8	11	17	2
N 9	18	12	0
N 10	16	13	1
N 11	17	12	1
N 12	18	11	1
N 21	12	18	0
N 23	12	18	0
N 27	12	17	1
Total	215	167	8
Average	55.1 %	42.8 %	2.1 %

It appears from the tables that there is a considerably larger number of polyploid cell nuclei in the squamous-cell and adenocarcinomata than in the small-celled anaplastic carcinoma. In each section, however, only 30–40 tumour cell nuclei were measured. In order to study the number of polyploid cell nuclei in greater detail, measurements were performed in tumour sections on all nuclei which seemed to have a DNA content close to or above twice the modal value per 1500–2000 cell nuclei. These nuclei are very large and dark. If the

Table 42. *Number of polyploid cell nuclei in squamous-cell carcinomata.*

Section No.	No. of cells counted	Polyploid cell nuclei No.	per 1000
E 1	1500	10	6.67
E 6	2000	3	1.50
E 9	2000	0	0.00
E 10	1500	7	4.67
E 11	1500	6	4.00
E 17	2000	11	5.50
E 24	1500	21	14.00
E 26	1500	10	6.67
E 28	1500	15	10.00
E 29	2000	4	2.00
E 32	1500	14	9.33
E 33	1500	13	8.67
E 34	1500	5	3.33
Average			5.87

Table 43. *Number of polyploid cell nuclei in adenocarcinomata.*

Section No.	No. of cells counted	Polyploid cell nuclei No.	per 1000
B 5	2000	3	1.50
B 6	2000	13	6.50
B 9	1500	24	16.00
B 11	2000	9	4.50
B 12	2000	7	3.50
B 13	2000	8	4.00
B 16	2000	13	6.50
B 18	1500	17	11.33
Average			6.73

Table 44. *Number of polyploid cell nuclei in small-celled anaplastic carcinomata.*

Section No.	No. of cells counted	Polyploid cell nuclei No.	per 1000
N 1	2000	0	0.00
N 4	2000	0	0.00
N 5	2000	0	0.00
N 6	2000	1	0.50
N 7	2000	0	0.00
N 8	2000	1	0.50
N 9	2000	2	1.00
N 10	2000	1	0.50
N 11	2000	4	2.00
N 12	2000	2	1.00
N 21	2000	0	0.00
N 23	2000	1	0.50
N 27	2000	4	2.00
Average			0.62

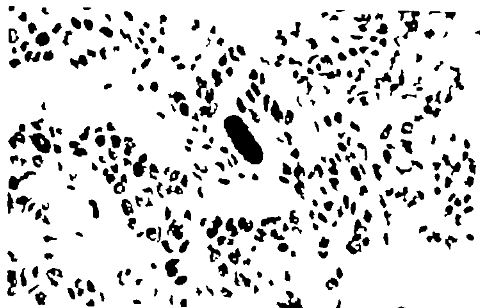


Fig. 39.—Polyploid cell nucleus. Squamous-cell carcinoma. (RNase gallocyanin-chromalum, × 350).



Fig. 40.—Polyploid cell nucleus. Adenocarcinoma. (RNase gallocyanin-chromalum, × 350)



Fig. 41.—Small-celled anaplastic carcinoma. (RNase gallocyanin-chromalum × 350)

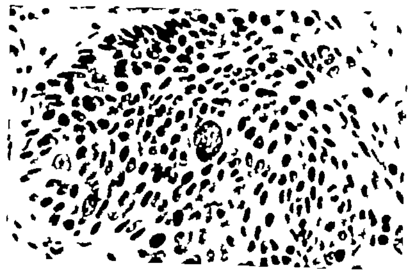


Fig. 42.—Polyploid cell nucleus. Squamous-cell carcinoma. (RNase, gallocyanin-chromalum, × 350).



Fig. 43.—Polyploid cell nucleus. Adenocarcinoma. (RNase, gallocyanin-chromalum, × 350).



Fig. 44.—Small-celled anaplastic carcinoma. (RNase, gallocyanin-chromalum, 350).



Fig. 39.—Polyploid cell nucleus. Squamous-cell carcinoma. (RNase gallocyanin-chromalum, × 350)



Fig. 40.—Polyploid cell nucleus. Adenocarcinoma. (RNase gallocyanin-chromalum, × 350)



Fig. 41.—Small-celled anaplastic carcinoma. (RNase gallocyanin-chromalum, × 350)

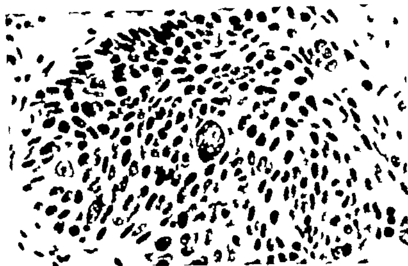


Fig. 42.—Polyploid cell nucleus. Squamous-cell carcinoma. (RNase, gallocyanin-chromalum, × 350)

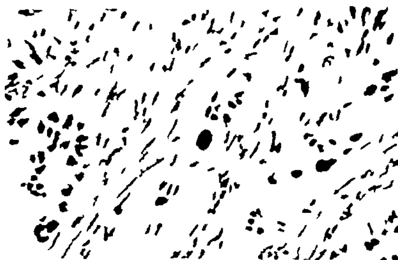


Fig. 43.—Polyploid cell nucleus. Adenocarcinoma. (RNase, gallocyanin-chromalum, × 350).



Fig. 44.—Small-celled anaplastic carcinoma. (RNase, gallocyanin-chromalum, × 350)



Fig. 45 —Polyploid cell nucleus. Squamous-cell carcinoma. (RNase gallocyanin chromalum, × 350)



Fig. 46 —Polyploid cell nucleus. Adenocarcinoma. (RNase gallocyanin-chromalum, 350).



Fig. 47 —Small-celled anaplastic carcinoma. (RNase gallocyanin-chromalum, 350).

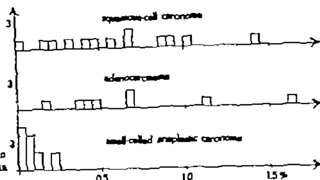


Fig. 48.—Number of polyploid cell nuclei in squamous-cell carcinomata, adenocarcinomata and small-celled anaplastic carcinomata.

measured DNA content was higher than 2 ½ × the modal value, they were recorded as polyploid cell nuclei (figs. 39–47)

The number of polyploid cell nuclei in the various types of carcinoma is stated in tables 42, 43 and 44

The distribution of the number of polyploid cell nuclei per 1000 is represented graphically in figure 48.

It appears that the number of polyploid cell nuclei in the squamous-cell carcinoma and in the adenocarcinoma was 10 times as large as in the small-celled anaplastic carcinoma. (The numbers of polyploid cell nuclei in the two statements, viz. tables 39–41 and tables 42–44 differ because the DNA content in the former case is calculated from the content of only 30 nuclei, and the number is stated in relation to that of non-sectioned, regular nuclei, while the number in the latter case, in order to obtain more comparable values, is stated for a considerably larger number of cells (1500–2000) whether the nuclei were sectioned or not.)

In the polyploid cell nuclei of the small-celled anaplastic carcinoma the DNA concentration was relatively close to twice the modal value, whereas the squamous-cell carcinoma and adenocarcinoma contained many nuclei with a considerably increased content of DNA, up to 10–11 times as much as the basal modal value.

Table 45 shows the DNA content of the polyploid cell nuclei in relation to the modal value.

Metastases

The material studied consisted of 34 biopsy specimens of bronchogenic carcinomata from 32 patients who were all subjected to mediastinoscopy (Jepsen 1966) A positive specimen with carcinomatous tissue from the mediastinal lymph nodes was obtained in 12 patients (squamous-cell carcinoma, 3 adenocarcinoma, 5 and small-celled anaplastic carcinoma, 4) Carcinomatous tissue from the metastases suitable for microphotometric measurements was

Table 45 *Number of polyploid cell nuclei in relation to the modal value*

DNA content/ modal value	No. of polyploid cell nuclei: Squamous-cell carcinoma	Adeno-carcinoma	Small-celled anaplastic carcinoma
2.5– 2.9	40	35	7
3.0– 3.4	30	18	6
3.5– 3.9	10	18	2
4.0– 4.4	12	6	–
4.5– 4.9	8	4	1
5.0– 5.4	3	1	–
5.5– 5.9	7	5	–
6.0– 6.4	2	1	–
6.5– 6.9	2	3	–
7.0– 7.4	1	2	
7.5– 7.9	1	–	–
8.0– 8.4	1	1	
8.5– 8.9		–	–
9.0– 9.4	1	–	–
9.5– 9.9	–	–	
10.0–10.4		–	–
10.5–10.9		–	–
11.0–11.4			–
11.5–11.9	1	–	–



Fig. 45 —Polyploid cell nucleus. Squamous-cell carcinoma (RNase gallocyanin-chromalum, × 350).



Fig. 46 —Polyploid cell nucleus. Adenocarcinoma. (RNase gallocyanin-chromalum, 350)



I g. 47 —Small-celled na plastic carcinoma (RN se gallocyan ch omalum, 350)

Table 48. *Cytoplasmic extinction in small-celled anaplastic carcinomata.*

Section No	1	2	3	Average	RNase-treated tumour tissue
N 1	0.22	0.20	0.22	0.21	0.03
N 4	0.16	0.15	0.14	0.15	0.03
N 5	0.12	0.12	0.14	0.13	0.03
N 6	0.20	0.19	0.17	0.19	0.04
N 7	0.12	0.11	0.10	0.11	0.02
N 8	0.10	0.09	0.10	0.10	0.03
N 9	0.17	0.15	0.16	0.16	0.03
N 10	0.10	0.10	0.11	0.10	0.03
N 11	0.13	0.13	0.12	0.13	0.04
N 12	0.19	0.14	0.15	0.16	0.03
N 21	0.16	0.15	0.20	0.17	0.03
N 23	0.14	0.12	0.12	0.13	0.03
N 27	0.11	0.11	0.10	0.11	0.03
Average				0.14	0.03

have any significant influence on the cytoplasmic extinction (see table 47) Tables 46, 47 and 48 show the results of the cytoplasmic measurements in the various types of carcinoma.

It appears that the cytoplasmic extinction, as a reflection of the concentration of RNA in the squamous-cell carcinomata, is considerably higher in normal bronchial epithelium, and of the same order as in the metaplastic bronchial epithelium. With the aforementioned reservation, the same applies to the adenocarcinomata. In addition, the cytoplasmic volume in these two types of carcinoma must be estimated to be considerably larger than in the basal cells of normal bronchial epithelium. The total cytoplasmic content of RNA in the squamous-cell carcinomata, and also in the adenocarcinomata, thus seems to be considerably higher than in normal epithelium. In the small celled anaplastic carcinoma, the cell nucleus is surrounded only by a very thin rim of cytoplasm, which is apparently not thicker than that of the basal cells in the normal bronchial epithelium. As the concentration of cytoplasmic RNA in this type of carcinoma is a little lower than in the basal cells of normal epithelium, this means that the cytoplasmic RNA content is at least not greater than in the cytoplasm of the basal cells of normal bronchial epithelium, and is considerably less than that present in metaplastic epithelium as well as in the squamous-cell carcinoma and the adenocarcinoma.

Conclusions

Nuclear measurements in squamous-cell carcinomata, adenocarcinomata and small-celled anaplastic carcinomata revealed a considerably increased average content of DNA in the nuclei as compared with the nuclei in normal bronchial epithelium, with a wide dispersion of the values from the modal value up to twice that level and even higher

The corrected average value of the DNA content was lower in the small-celled anaplastic carcinoma than in the squamous-cell carcinoma and the adenocarcinoma. The relation, if any between the nucleic acid content in the bronchogenic carcinomata and the prognosis of these tumours thus seems to be that the most malignant type, the small-celled anaplastic carcinoma, on an average contains a smaller amount of DNA per *nucleus* than the less malignant squamous-cell carcinoma and adenocarcinoma.

The modal value of the tumour stem cells was within the diploid and near-diploid range or within the triploid and near-triploid range, without any difference between the various types of carcinomata.

Polyploid cell nuclei occurred with a considerably higher frequency in squamous-cell carcinoma and adenocarcinoma than in the highly malignant small-celled anaplastic carcinoma. This may mean that the two first mentioned tumour types provide less favourable growth conditions causing inhibition of mitosis, while the cells continue to synthetize DNA, resulting in the formation of large, dark nuclei with a high content of DNA.

The considerably greater malignancy of the small-celled anaplastic carcinoma is due to its greater tendency to rapid growth and rapid metastatic spread, and to its invasion of the

available only in one case (E 26). In this case, an average DNA content of the same order as in the primary tumour was found, and the distribution of the cell nuclei in the various classes and the number of polyploid cell nuclei were also as in the primary tumour (6.0 and 6.7 per thousand, respectively).

Of the 32 patients, three with squamous cell carcinoma and one with small-celled anaplastic carcinoma were alive after five years without signs of recurrence (the case of anaplastic carcinoma was accidentally revealed by *routine radiography*; *the tumour* was very small and produced no symptoms). The average survival times for the patients who died after the diagnosis had been made were for squamous cell carcinoma, adenocarcinoma and small celled anaplastic carcinoma 7.8 months, 3.9 months and 3.6 months, respectively.

A correlation between the number of polyploid cell nuclei in the carcinomata and the survival time of the patients cannot be established as the series is too small to allow any definite conclusions for that purpose.

Cytoplasmic Measurements

The average cytoplasmic extinction was determined in three areas in each tumour section. In each area, 50 measurements were performed with one or two measurements of the extinction on each tumour cell. In many sections of the adenocarcinomata, areas with PAS-positive mucin were present, giving a metachromatically red colour with gallocyanin-chromalum. In the measurements of the cytoplasmic extinction these areas with much PAS-positive material were avoided. It was, however, not possible in all cases completely to exclude absorption of metachromatically stained mucin in the adenocarcinomata. The measurements of the cytoplasmic extinction in this group of carcinomata must therefore be taken with some reservation.

The cytoplasmic measurements were all performed at the absorption maximum of nucleic acids, 570 nm. The absorption curve of the metachromatically stained mucin was determined in one RNase-treated gallocyanin-chromalum-stained section. The absorption maximum was found to be 540 nm, and at a wave length of 570 nm the absorption was only three quarters of that at the maximum. This contributes to a reduction in the cytoplasmic absorption of mucin at 570 nm. In one adenocarcinoma specimen (B 18) the tissue available was insufficient for cytoplasmic measurements on gallocyanin-chromalum stained sections.

RNase treatment had removed the cytoplasmic RNA from the sections, and only in the adenocarcinomata there remained a weak, metachromatically red colour which did not

Table 46 *Cytoplasmic extinction in squamous cell carcinomata*

Section No.	1	[illegible]	3	Average	RNase-treated tumour tissue
E 1	0.24	0.19	0.[illegible]2	0.[illegible]	0.05
E 6	0.20	0.19	0.18	0.19	0.04
E 9	0.24	0.[illegible]6	0.[illegible]3	0.24	0.05
E 10	0.25	0.19	0.19	0.[illegible]1	0.03
E 11	0.28	0.23	0.2[illegible]	0.[illegible]5	0.03
E 17	0.[illegible]8	0.[illegible]8	0.33	0.30	0.04
E 4	0.[illegible]5	0.[illegible]2	0.26	0.24	0.04
E 6	0.[illegible]7	0.[illegible]7	0.[illegible]5	0.27	0.04
E 8	0.[illegible]8	0.31	0.31	0.30	0.03
E 29	0.23	0.25	0.[illegible]3	0.24	0.03
E 32	0.30	0.32	0.31	0.31	0.03
E 33	0.[illegible]7	0.33	0.27	0.[illegible]9	0.04
E 34	0.[illegible]6	0.2[illegible]	0.[illegible]3	0.[illegible]4	0.03
Average				0.[illegible]5	0.04

Table 47 *Cytoplasmic extinction in adenocarcinomata*

Section No.	1	[illegible]	3	Average	RNase-treated tumour tissue
B 5	0.[illegible]0	0.[illegible]3	0.20	0.[illegible]1	0.07
B 6	0.[illegible]	0.[illegible]4	0.[illegible]3	0.[illegible]3	0.04
B 9	0.30	0.41	0.35	0.35	0.04
B 11	0.[illegible]1	0.[illegible]6	0.29	0.[illegible]5	0.04
B 1[illegible]	0.[illegible]	0.[illegible]6	0.25	0.[illegible]4	0.03
B 13	0.33	0.[illegible]3	0.[illegible]7	0.28	0.04
B 16	0.27	0.[illegible]3	0.[illegible]	0.[illegible]4	0.04
B 18	–	–	–	–	0.03
Average				0.[illegible]6	0.04

Table 48. *Cytoplasmic extinction in small-celled anaplastic carcinomata.*

Section No.	1.	2.	3	Average	RNase-treated tumour tissue
N 1	0.22	0.20	0.22	0.21	0.03
N 4	0.16	0.15	0.14	0.15	0.03
N 5	0.12	0.12	0.14	0.13	0.03
N 6	0.20	0.19	0.17	0.19	0.04
N 7	0.12	0.11	0.10	0.11	0.02
N 8	0.10	0.09	0.10	0.10	0.03
N 9	0.17	0.15	0.16	0.16	0.03
N 10	0.10	0.10	0.11	0.10	0.03
N 11	0.13	0.13	0.12	0.13	0.04
N 12	0.19	0.14	0.15	0.16	0.03
N 21	0.16	0.15	0.20	0.17	0.03
N 23	0.14	0.12	0.12	0.13	0.03
N 27	0.11	0.11	0.10	0.11	0.03
Average				0.14	0.03

have any significant influence on the cytoplasmic extinction (see table 47). Tables 46, 47 and 48 show the results of the cytoplasmic measurements in the various types of carcinoma.

It appears that the cytoplasmic extinction, as a reflection of the concentration of RNA in the squamous-cell carcinomata, is considerably higher in normal bronchial epithelium, and of the same order as in the metaplastic bronchial epithelium. With the aforementioned reservation, the same applies to the adenocarcinomata. In addition, the cytoplasmic volume in these two types of carcinoma must be estimated to be considerably larger than in the basal cells of normal bronchial epithelium. The total cytoplasmic content of RNA in the squamous-cell carcinomata, and also in the adenocarcinomata, thus seems to be considerably higher than in normal epithelium. In the small-celled anaplastic carcinoma, the cell nucleus is surrounded only by a very thin rim of cytoplasm, which is apparently not thicker than that of the basal cells in the normal bronchial epithelium. As the concentration of cytoplasmic RNA in this type of carcinoma is a little lower than in the basal cells of normal epithelium, this means that the cytoplasmic RNA content is at least not greater than in the cytoplasm of the basal cells of normal bronchial epithelium, and is considerably less than that present in metaplastic epithelium as well as in the squamous-cell carcinoma and the adenocarcinoma.

Conclusions

Nuclear measurements in squamous-cell carcinomata, adenocarcinomata and small-celled anaplastic carcinomata revealed a considerably increased average content of DNA in the nuclei as compared with the nuclei in normal bronchial epithelium, with a wide dispersion of the values from the modal value up to twice that level and even higher.

The corrected average value of the DNA content was lower in the small-celled anaplastic carcinoma than in the squamous-cell carcinoma and the adenocarcinoma. The relation, if any, between the nucleic acid content in the bronchogenic carcinomata and the prognosis of these tumours thus seems to be that the most malignant type, the small-celled anaplastic carcinoma, on an average contains a smaller amount of DNA per nucleus than the less malignant squamous-cell carcinoma and adenocarcinoma.

The modal value of the tumour stem cells was within the diploid and near-diploid range, or within the triploid and near-triploid range, without any difference between the various types of carcinomata.

Polyploid cell nuclei occurred with a considerably higher frequency in squamous-cell carcinoma and adenocarcinoma than in the highly malignant small-celled anaplastic carcinoma. This may mean that the two first-mentioned tumour types provide less favourable growth conditions causing inhibition of mitoses, while the cells continue to synthetize DNA, resulting in the formation of large dark nuclei with a high content of DNA.

The considerably greater malignancy of the small-celled anaplastic carcinoma is due to its greater tendency to rapid growth and rapid metastatic spread, and to its invasion of the

available only in one case (E 26) In this case, an average DNA content of the same order *as in the primary tumour was found, and the* distribution of the cell nuclei in the various classes, and the number of polyploid cell nuclei, were also as in the primary tumour (6 0 and 6 7 per thousand respectively)

Of the 32 patients, three with squamous cell carcinoma and one with small-celled anaplastic carcinoma were alive after five years without signs of recurrence (the case of anaplastic carcinoma was accidentally revealed by *routine radiography the tumour was very small* and produced no symptoms) The average survival times for the patients who died after the diagnosis had been *made were for squamous*-cell carcinoma, adenocarcinoma and small celled anaplastic carcinoma 7 8 months 3 9 months and 3 6 months, respectively

A *correlation* between the number of polyploid cell nuclei in the carcinomata and the survival time of the patients cannot be established as the series is too small to allow any definite conclusions for that purpose.

Cytoplasmic Measurements

The average cytoplasmic extinction was determined in three areas in each tumour section. In each area, 50 measurements were performed with one or two measurements of the extinction on each tumour cell In many sections of the adenocarcinomata, areas with PAS-positive mucin were present, giving a metachromatically *red colour with gallocyanin* chromalum In the measurements of the cytoplasmic extinction these areas with much PAS-positive material were avoided. It was, however not possible in all cases completely to exclude absorption of metachromatically stained mucin in the adenocarcinomata. The measurements of the cytoplasmic extinction in this group of carcinomata must therefore be taken *with some reservation.*

The cytoplasmic measurements were all performed at the absorption maximum *of nucleic* acids, 570 nm The absorption curve of the metachromatically stained mucin was determined in one RNase-treated gallocyanin-chromalum-stained section The absorption *maximum was found to be 540* nm and at a wave length of 570 nm the absorption was only three quarters of that at the maximum. This contributes to a reduction in the cytoplasmic absorption of mucin at 570 nm In one adenocarcinoma specimen (B 18) the tissue available was insufficient for cytoplasmic measurements on gallocyanin-chromalum stained sections.

RNase treatment had removed the cytoplasmic RNA from the sections, and only in *the adenocarcinomata there remained a weak* metachromatically red colour which did not

Table 46 *Cytoplasmic extinction in squamous cell carcinomata*

Section No.	1	2	3	Average	RNase-treated tumour tissue
E 1	0.24	0.19	0.22	0.[illegible]2	0.05
E 6	*0.20*	*0.19*	*0.18*	*0.19*	*0.04*
E 9	0.24	0.26	0.[illegible]3	0.[illegible]4	0.05
E 10	0.25	0.19	0.19	0.[illegible]1	0.03
E 11	0.28	0.23	0.2[illegible]	0.25	0.03
E 17	0.28	0.28	0.33	0.30	0.04
E 24	0.25	0.22	0.26	0.24	0.04
E 26	0.27	0.27	0.[illegible]5	0.27	0.04
E 28	0.28	0.31	0.31	0.30	0.03
E 29	*0.23*	*0.25*	*0.23*	*0.24*	*0.03*
E 32	0.30	0.32	0.31	0.31	0.03
E 33	0.27	0.33	0.[illegible]7	0.29	0.04
E 34	0.[illegible]6	0.22	0.23	0.24	0.03
Average				0.25	0.04

Table 47 *Cytoplasmic extinction in adenocarcinomata*

Section No.	1	2	3	Average	RNase-treated tumour tissue
B 5	0.[illegible]0	0.23	0.[illegible]0	0.21	0.07
B 6	0.2[illegible]	0.[illegible]4	0.21	0.[illegible]3	0.04
B 9	0.30	0.41	0.35	0.35	0.04
B 11	0.21	0.26	0.29	0.25	0.04
B 1	*0.[illegible]*	0.[illegible]6	0.[illegible]	0.[illegible]4	0.03
B 13	0.33	0.23	0.[illegible]7	0.[illegible]	0.04
B 16	0.27	0.[illegible]3	0.2	0.[illegible]4	0.04
B 18	–	–	–	–	0.03
Average				0.[illegible]6	0.04

SUMMARY

Chapter 1 gives a survey of the chemistry localization and function of nucleic acids. Histological methods for the demonstration of nucleic acids are discussed, and special mention is made of staining with gallocyanin-chromalum, including the theoretical background of the staining method. The advantages of this method in connection with photometric measurements are emphasized. The use of RNase in histological studies, especially of formalin-fixed tissue, is discussed. Furthermore, a survey is given of photometric investigations of histological sections, especially in visible light.

Chapter 2 contains a description of the series of bronchial biopsy specimens, fixation, RNase treatment and staining of the sections, and also of photometric measurements of the nuclear and cytoplasmic nucleic acid content of the epithelial tissue. The absorption curves for gallocyanin-chromalum in solution and bound to nucleic acids in bronchial epithelial nuclei were determined. It was shown that the absorption maxima were identical, whereas the curve for the tissue-bound gallocyanin-chromalum was at a higher level than that for gallocyanin-chromalum in solution, both within the long-wave and the short-wave range. The photometric measuring errors were determined in nuclei and cytoplasm.

Chapter 3 is concerned with the nucleic acid content in normal nuclei and the concentration of cytoplasmic RNA in various tissues.

In the basal cells of morphologically normal bronchial epithelium, the nucleic acid content was measured in nuclei and cytoplasm. No correlation was found between the nucleic acid content and the ages of the patients, diagnosis or smoking habits. No difference was revealed in the nucleic acid content of morphologically normal bronchial epithelium in a control group and in a group of patients with bronchogenic carcinoma elsewhere in the bronchial system.

The content of RNA of normal nuclei in the bronchial epithelium was calculated after measuring the nuclear nucleic acid content without and with RNase treatment. The nuclear RNA content was 1–6 % of the total nucleic acid content of the cell nucleus.

Methods for the determination of the mitotic activity are reviewed in *Chapter 4*. In the study of the nucleic acid content of bronchial epithelium a method was elaborated for the determination of the mitotic activity on the basis of photometric measurements of the DNA content of the cell nuclei. This method has proved to be suitable for relative determinations of the mitotic activity in bronchial epithelium.

On the basis of theoretical calculations of the distribution of DNA-synthetizing cells it was shown that the absolute number of mitoses in the bronchial epithelium can be estimated.

No difference was revealed in the mitotic activity of normal bronchial epithelium in a control group and in a group of patients with bronchogenic carcinoma elsewhere in the bronchial system. Correlated with the investigations in Chapter 3 this implies that in morphologically normal bronchial epithelium no difference can be demonstrated in the nucleic acid content in nuclei and cytoplasm, or in the mitotic activity in a control group and in a group of patients with bronchogenic carcinoma elsewhere in the bronchial system.

In *Chapter 5* the histological structure of the

pulmonary vessels. If the polyploid cells can be taken as a manifestation of unfavourable growth conditions and a lower malignancy it must be assumed that only relatively slight inhibition of the growth occurs in the small-celled anaplastic carcinoma as compared with the squamous-cell carcinoma and adenocarcinoma, which is in good agreement with clinical observations

The presence of polyploid cell nuclei in the bronchial epithelial tissue is nearly always suggestive of a malignant tumour since the only case with a polyploid cell nucleus without manifest malignancy was one of atypical metaplasia. On the other hand, the absence of polyploid cell nuclei does not exclude a malignant tumour because the small-celled anaplastic carcinoma contains only a few or no polyploid cell nuclei

Cytoplasmic measurements revealed a higher concentration and probably a higher content of cytoplasmic RNA in the squamous-cell and adenocarcinomata than in the basal cells of normal bronchial epithelium However the content of cytoplasmic RNA in the small-celled anaplastic carcinoma is probably of the same order as in the basal cells of normal epithelium, possibly lower

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RESUMÉ

I *kapitel 1* gives en oversigt over nucleinsyrernes kemi, lokalisation og funktion. Histologiske metoder til påvisning af nucleinsyrer diskuteres, og specielt omtales farvning med gallocyanin-chromalun og den teoretiske baggrund for farvemetoden. Dens fordele ved fotometriske målinger fremhæves. Anvendelsen af ribonuclease ved histologiske undersøgelser og især på formalinfixeret væv diskuteres. Endvidere gives en oversigt over fotometriske undersøgelser af histologiske præparater specielt i synligt lys.

I *kapitel 2* omtales materialet af bronchiebiopsier fixering, RNase behandling og farvning af materialet, og endvidere fotometriske målinger af nucleinsyreindholdet i kerner og cytoplasma i det epitheliale væv. Absorptionskurven for gallocyanin-chromalun i opløsning og bundet til nucleinsyre i bronchieepithelkerner blev bestemt. Det blev fundet, at absorptionsmaximum var det samme, medens kurven for det vævsbundne gallocyanin-chromalun lå højere end kurven for gallocyanin-chromalun i opløsning, både i det langbølgede og navnlig i det kortbølgede område. De fotometriske målefejl blev bestemt for bronchieepithelet i kerner og i cytoplasma.

I *kapitel 3* gives en oversigt over nucleinsyre indholdet i normale kerner og koncentrationen af cytoplasmatisk RNA i forskelligt væv.

I de basale celler i det morfologisk normale bronchieepithel blev nucleinsyreindholdet målt i kerner og i cytoplasma. Der blev ikke fundet nogen relation mellem nucleinsyreindhold og alder diagnose eller tobaksforbrug. Der blev ikke fundet nogen forskel mellem nucleinsyreindhold i morfologisk normalt bronchieepithel i en kontrolgruppe og i en patientgruppe med bronchogent carcinom et andet sted i bronchieepithelet.

Indholdet af RNA i normale kerner i bronchieepithelet blev beregnet efter måling af kernenucleinsyreindholdet uden og med RNase behandling. Kerne RNA indholdet var 1–6 % af kernens totale nucleinsyreindhold.

Metoder til bestemmelse af mitoseaktiviteten gennemgås i *kapitel 4*. Under undersøgelse af nucleinsyreindholdet i bronchieepithel, er der udarbejdet en metode til bestemmelse af mitoseaktiviteten på basis af fotometriske målinger af cellekernernes DNA indhold. Denne metode har vist sig velegnet til relative bestemmelser af mitoseaktiviteten i bronchieepithelet.

Efter teoretiske beregninger over fordelingen af DNA-synthetiserende celler kan det vises, at man til en vis grad også kan bedømme det absolutte antal mitoser i bronchieepithelet.

Der blev ikke fundet nogen forskel i mitoseaktiviteten i normalt bronchieepithel i en kontrolgruppe og i en patientgruppe med bronchogent carcinom et andet sted i bronchieepithelet. Sammenholdt med undersøgelserne i kapitel 3 vil det sige, at der ikke i morfologisk normalt bronchieepithel kan påvises nogen forskel i nucleinsyreindhold i kerner og i cytoplasma, eller i mitoseaktivitet hos en kontrolgruppe og en patientgruppe, som har udviklet bronchogent carcinom et andet sted i bronchieepithelet.

I *kapitel 5* beskrives det histologiske billede af metaplastiske forandringer i bronchieepithelet.

Ved forskellige grader af metaplasi blev der som udtryk for en forøget mitoseaktivitet, gennemsnitlig fundet et ca. 10 gange så stort antal hyperdiploide kerner som i det normale bron-

metaplastic alterations in the bronchial epithelium is described

In various degrees of metaplasia, the increased mitotic activity was reflected in an increase in the number of hyperdiploid nuclei, up to an average of about 10 times as many as in normal bronchial epithelium. In addition, an increased concentration of RNA in the cytoplasm was found Only when morphological changes such as metaplasia, occur in the bronchial epithelium can alterations in the nucleic acid content of the bronchial epithelium be disclosed, either in the form of an increased number of hyperdiploid nuclei or as an increased concentration of RNA in the cytoplasm.

In Chapter 6 a survey is given of the pathological anatomy of the bronchial adenoma and of the nucleic acid content and chromosome constitution in benign tumours The DNA content of the tumour stem line, the modal value was determined on the basis of the DNA content of normal epithelial nuclei or lymphocytes in the sections, and it was demonstrated that the modal value, apart from one exception within the triploid range, was within the diploid or near-diploid range. The number of hyperdi*ploid nuclei was three times as high as in nor*mal bronchial epithelium, while the cytoplasmic RNA concentration with a single exception, was as in the normal bronchial epithelium. Only in one case was the RNA concentration in the cytoplasm increased. Unlike the bronchogenic carcinomata, there was only a slight dispersion of the DNA content in the nuclei and unlike the carcinomata no values above twice the basal modal value were found

In Chapter 7 the classification of the bronchogenic carcinomata and factors responsible for the prognosis are mentioned. Furthermore a survey is given of *the DNA content and the* chromosomes of tumour cells The formation and the significance of the polyploid cell nuclei are discussed The measurements of the DNA content in bronchogenic carcinomata revealed a considerably increased average DNA content as compared *with normal nuclei, with a wide* dispersion of the values from the modal value up to twice that level and even higher

The average DNA content in tumour-cell nuclei was lower in the most malignant type, the small-celled anaplastic carcinoma than in the squamous-cell carcinoma and the adenocarcinoma.

No difference in the position of the modal value in the various types of carcinomata was found An analysis of the distribution of the *DNA values in bronchogenic tumours showed* that 10 times as many polyploid cell nuclei were present in squamous-cell and adenocarcinomata as in the small-celled anaplastic carcinoma. This suggests the existence of more unfavourable growth conditions in the first two tumours, resulting in inhibition of mitosis and formation of polyploid cell nuclei The occurrence of polyploid cell nuclei is nearly always suggestive of a malignant tumour On the other hand the absence of polyploid cell nuclei does *not exclude a bronchogenic carcinoma* because the small-celled anaplastic carcinoma contains only a few or no polyploid cell nuclei.

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chieepithel Endvidere blev der fundet en forøget koncentration af RNA i cytoplasmaet. Først når der optræder morfologiske ændringer i bronchieepithelet i form af metaplasi, kan der således påvises ændringer i bronchieepithelets nucleinsyreindhold, dels i form af et forøget antal hyperdiploide kerner dels i form af en forøget koncentration af RNA i cytoplasmaet.

I *kapitel 6* gives en oversigt over bronchial adenomets pathologiske anatomi og over nucleinsyreindhold og kromosomforhold i benigne tumorer DNA indholdet i tumorernes stamlinie, modalværdien, blev fastlagt på basis af DNA indholdet i normale epitheliale kerner eller i lymfocyter i præparaterne og det blev fundet, at modalværdien, med én undtagelse i det triploide område lå i det diploide eller nær-diploide område Antallet af hyperdiploide kerner var 3 gange så stort som i det normale bronchieepithel medens den cytoplasmatiske RNA koncentration med en enkelt undtagelse var som i det normale bronchieepithel. Kun i ét tilfælde var RNA koncentrationen i cytoplasmaet forhøjet. Til forskel fra de bronchogene carcinomer var der kun en ringe spredning i kernernes DNA indhold, og til forskel fra carcinomerne ingen værdier over det dobbelte af den basale modale værdi

I *kapitel 7* omtales klassificationen af de bronchogene carcinomer og de faktorer som har betydning for prognosen. Endvidere gives en oversigt over DNA indhold og kromosomer i tumorceller Dannelsen og betydningen af polyploide cellekerner omtales. Resultatet af målingerne af DNA indholdet i de bronchogene carcinomer viste et i forhold til normale kerner betydeligt forhøjet gennemsnitligt DNA indhold med en stor spredning af værdierne fra modalværdien til det dobbelte heraf og endnu højere

Den gennemsnitlige værdi af DNA indholdet i tumorcellekernerne var lavere i den mest maligne type, det småcellede anaplastiske carcinom, end i det planocellulære carcinom og adenocarcinomet.

Der blev ikke fundet nogen forskel i modalværdiens beliggenhed i de forskellige carcinomtyper Ved analyse af fordelingen af DNA værdierne i de bronchogene tumorer viste det sig, at der fandtes 10 gange så mange polyploide cellekerner i det planocellulære carcinom og i adenocarcinomet som i det småcellede anaplastiske carcinom. Det kan betyde, at der i de to førstnævnte tumorer er mere ugunstige vækstbetingelser som gør at mitoserne hæmmes, og at der dannes polyploide cellekerner Forekomsten af polyploide cellekerner i bronchieepithelet vil næsten altid betyde en malign tumor medens omvendt mangelen på polyploide cellekerner ikke udelukker et bronchogent carcinom, idet det småcellede anaplastiske carcinom kun indeholder få eller ingen polyploide cellekerner

Den cytoplasmatiske koncentration af RNA blev fundet højere i det planocellulære carcinom og i adenocarcinomet end i det normale bronchieepithel. I det småcellede anaplastiske carcinom er der kun en smal cytoplasmabræmme omkring kernen med en lav koncentration af RNA. Medens normale celler i intensiv vækst eller med stor aktivitet har et højt indhold af cytoplasmatisk RNA afspejler den høje malignitetsgrad af det småcellede anaplastiske carcinom sig således ikke i et højere indhold af RNA i cytoplasmaet.

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CONTENTS

Abstract

Theories of hearing so far available cannot elucidate the numerous physical and medical observations regarding the hearing instrument. The main difficulty is presented by the cochlear partition which has been considered as one part, in spite of the fact that it consists of a great number of very precisely built and arranged parts. Therefore, detailed investigations were carried out on the ear of *Anoa buballs* which is very similar to that of man, and it was possible to obtain a clear and complete picture of the organ as a whole and of the function and interaction of its individual parts, and to develop a theory of hearing which explains numerous observations on hearing on a physical basis, and without requiring far-fetched arguments or obscure mechanisms.

The experiments permit representation of the cochlear partition as a cybernetic model of a highly involved frequency analysing instrument which may be briefly described as follows.

Oscillations of the endolymph are picked up by those narrow oblique segments of the tectorial membrane which possess corresponding natural frequencies. Due to the many oriented reinforcing fibrils the tectoria has anisotropic oscillator properties and its segments behave like the reeds of a reed-type frequency meter

The tectoria is connected to the stereocilia which bridge the gap to the reticular lamina. These transmitter pins act on the cuticular plate and this diaphragm piston displaces the cytoplasm of the transducers and strains the fine nerve endings at their lower side.

The transducers are mounted in a lattice girder The lower rigid plate of this girder (basilar membrane) supports the lower side of the transducers by means of the phalangeal cells, and the upper plate (reticular lamina) holds the upper rim of the transducers. These plates are connected by rows of pillars towards their center At the edges they are supported by border cells and cells of Hensen. The outer transducer rows are shifted relative to the preceding ones and this angle of shift corresponds to the oblique arrangement of the reinforcing fibers within the tectorial membrane This enhances the precision of the instrument. Tuning of first and of second order neurons and behaviour of synaptic connections improve the analysing properties of the described instrument further

The experiments established 132 essential dimensions of parts as function of the distance of the place of measurement from the stapes. In a detailed discussion it is shown that the theory is capable of explaining the essential phenomena of hearing.

Introduction

In spite of concerted research efforts of physicists, physiologists, histologists, biochemists, and ear nose, and throat experts for at least one-and-a-half centuries, the physical mode of functioning of the inner ear still remains practically unsolved.

Many theories have been proposed to account for the phenomena of hearing and have proliferated principally because the location of the inner ear makes *intra vita* observations difficult, and, due to the very small size of the various parts of the inner ear they are very difficult to measure, especially as they are rather susceptible to damage during experiments.

In view of the shortage of sufficient physical data on the inner ear and on the translation of mechanical processes into neural stimuli it is not surprising that one has sometimes even resorted to proposing obscure mechanisms to explain the hearing process.

This, however does not appear to be a desirable approach, but one should try to obtain a clear picture of the inner ear as a physical instrument. As until now the organ of Corti has been treated as one unit when considering its mode of action—in spite of its very complex design—and since only insufficient information was available on the performance and interaction of the individual parts of the organ of Corti and on the dimensional changes of the parts between the stapes and the helicotrema, it was decided to try to obtain such data by experiment. Such data would produce a detailed "workshop-drawing" of the inner ear which in turn would allow us to see whether the data obtained would be compatible with a working hypothesis on the functioning of the inner ear derived earlier by theoretical reasoning. At any rate, a complete physical picture of the inner ear was expected to result in a better understanding of the organ.

Before going into details of experiments and results a brief account of the structure of the ear is given for the benefit of the reader not specialized in this line, and because these lines contain some new observations.

Construction of the Ear System and its Working

The following description will of necessity be brief For a detailed account it is suggested to refer to papers mentioned in the text.

Dimensional data is compiled in Table I. The individual main parts of the ear system are shown in Fig. 1

(1) *The Outer Ear* Contrary to most mammalia the human pinna (cf. Fig. 1) has lost most of its movability It is generally believed that its purpose is to collect sound and to help to locate the direction of its source.

Observations in connection with this investigation show however that the pinna also serves to improve the sensitivity of the hearing system in a more general way For instance, if one rubs the ridge of the nose very gently with the tip of a finger normally no noise is perceived. If one puts on, however spectacles in such a way that the sides of their frame rest against the pinnae but do not touch the temporal bone, a repetition of the experiment produces a distinct noise. The vibrations caused by rubbing the finger tip on the ridge of the nose are transmitted by the sides of the frame to the pinnae. The pinnae are internally reinforced by cartilage, and this cartilage picks up the vibrations and passes them on to the hearing apparatus. A similar difference is found if one rubs gently the pinnae as compared to the temporal bone.

But sound waves, too, will cause vibrations of the pinnae, which are then transmitted on as tissue-conducted sound (Körperschall) Therefore, the pinnae will pick up all vibrations which reach them. The efficiency of the pinnae regarding improvement of sensitivity of the ear in general will depend on many factors. Firstly on their size because this will determine the area which is in a position to pick up sound energy from the oscillating air particles. Secondly movability will play a role in mammalian pinnae because it permits the animal to adjust the pinnae in such a way to the oncoming sound waves that a maximum area is exposed to the waves. Then rigidity of reinforcing cartilage will determine efficiency because, e.g. very soft ears (i.e. where the pinna is not equipped with strong internal cartilage), will not transmit the vibration onward to the inner ear as well as others which are well reinforced.

The shape of the outer ear is a further factor because the properly shaped auricle (as, e.g. in some species of bats) will act like an acoustic horn and funnel the sound energy into the external auditory meatus. Finally the direction of the sound waves impinging on the auricle will influence the efficiency in particular if the pinna is not movable, as in man.

It is felt, therefore, that the action of the mammalian pinnae goes beyond the simple sound wave collector action of a funnel.

It is known that the skull also picks up sound energy which is then transmitted onward to the inner ear However the pinnae are far more efficient for physical reasons, discussion of which is beyond the scope of this paper

From the pinna a tapering canal (external auditory meatus) of about 2.7 cm length leads to the drum (membrana tympani). This is a *conical membrane of about 0 1 mm thickness*, which is reinforced by many internal fibers. The external auditory meatus has a natural frequency of about 3 000 cps. The transmission characteristics of the external auditory meatus and of the membrana tympani have been extensively studied (1–21 et al) The pneumatic form of sound energy is transformed to mechanical energy at the drum.

(2) *Middle Ear* To the other side of the drum is attached the handle of the hammer (malleus). This hammer is connected to the anvil (incus), which in turn acts on the stirrup (stapes). As the handle of the hammer is longer

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Table I

Item		Human ear[a]			*Anoa bubalis* (mean of 5 specimens)			Symbol (cf Fig. 4)	Remarks
		Min.	Average	Max.	Min.	Average	Max.		
Auditory meatus									
Cross section	(cm²)	0.3		0.5	0.37		0.8	*a* and *a*	Greater difference is due to greater length (horn effect)
Diameter	(cm)		0.7			1.0		*b*	No influence
Length	(cm)		2.7			7.0		*c*	Lower natural frequency of buffalo's external auditory meatus
Volume	(cm³)		1.0			4.1		*d*	No influence
Tympanic membrane									
Area	(cm²)	0.5		0.9	0.8		1.0	*e*	Less variation in area of buffalo's drums
Max. diameter	(mm)		ca. 10			ca. 12		*f*	The greater diameter corresponds to greater malleus length
Min. diameter	(mm)		ca. 8.5			ca. 8		*g*	
Thickness	(mm)		ca. 0.1			ca. 0.11		*h*	
Middle ear									
Malleus weight	(mg)		23			ca. 28		*i*	As malleus is suspended at center of gravity, therefore, no influence
Incus length	(mm)	5.5		6.0	6.6		7.8	*j*	Cf. remark under *f*
Incus weight	(mg)		27			ca. 76		*k*	Cf. remark under *i*
Stapes weight	(mg)		2.5			ca. 2.6		*l*	
length of footplate	(mm)		3.2			3.0		*m*	Footplate of buffalo more roundish than human one
width of footplate	(mm)		1.4			1.6		*n*	Cf. remark under *m*
area of footplate	(mm²)		3.2			3.4		*o*	Considering, *e*, *f* and *o*, overall transmission rather similar
Cochlea									
Length of cochlea duct	(mm)		35			36		*p*	Good agreement between ear of man and buffalo
Height of ducts	(mm)		ca. 1		0.4		1.2	*q*	No exact values for human ear. Influence negligible
Round window area	(mm²)		2			2.2			
Basilar membrane: width at stapes	(mm)		0.04			ca. 0.05		*s* }	Good agreement between width of basilar membrane and width ratio for man and *Anoa bubalis*
width at apex	(mm)		0.5			ca. 0.6		*t* }	
Helicotrema area	(mm²)	0.25		0.4	0.2		0.45	*u*	No influence
Max. diameter of coch.	(mm)		ca. 7		6.5		7.2	*v*	Good agreement
Height of cochlea	(mm)		ca. 6			ca. 6		*w*	Good agreement

[a] Data for human ear from refs. 25, 93–95.

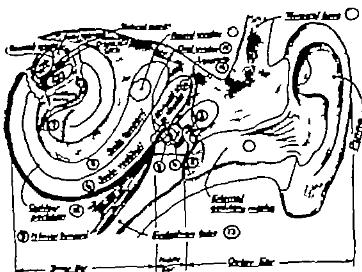


Fig. 1. Schematic diagram of human ear

(A) *Outer Ear*: Pinna
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Drum (membrana tympani) (2)

(B) *Middle Ear*
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Stirrup (Stapes) (5)
Eustachian tube (13)
Ligaments holding the ossicles (16)
Musculus tensor tympani (9)
Musculus stapedius (10)

(C) *Inner Ear*
Scala vestibuli (6)
Helicotrema (7)
Scala tympani (8)
Round window (11)
Oval window (15)
Cochlear partition (12)

The borders of the above sections of the ear have been indicated on the lower side of the drawing. The cochlear partition is shown once more in detail in the upper left corner. This represents a cross section through one semi turn of the cochlea. The three ducts (scala vestibuli, ductus cochlearis and scala tympani seperated by Reissner membrane and organ of Corti respectively) are clearly seen. The basilar membrane is on the lower side of the organ of Corti.

than the handle of the anvil (ratio ca. 1 : 1.3), the amplitudes of vibration are reduced. The footplate of the stapes is, furthermore, substantially smaller (ca. 3.2 mm²) than the active area of the drum (ca. 55 mm²). This leads to an increase of pressure at the footplate. This footplate is mounted into the oval window (fenestra ovalis) by means of an elastic ligament. It therefore moves like a piston, or as the movement is not exactly linear but somewhat rotational, rather like a flap. The footplate imparts the oscillations to the liquid (perilymph) in the scala vestibuli.

Malleus and Incus are attached to the walls of the cavity of the middle ear (cavum tympani) by ligaments, which are so arranged that these ossicles are suspended through their center of gravity, which enhances their transmission characteristics.

The middle ear ends at the footplate. Also the transmission characteristics of the middle ear are well known since long time (5, 19, 23–27 et al.)

The cavity of the middle ear is connected through the Eustachian tube to the rear part of the throat. This tube serves to keep pressure inside the middle ear equal to the outside pressure. To achieve this the Eustachian tube may be opened by tensioning certain muscles, e.g. the musculus tensor veli palatini, during the act of swallowing, or voluntarily.

To the handle of the hammer a muscle is

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Helicotrema area	(mm^2)	0.[illegible]5		0.4	0.[illegible]		0.45	*u*	No influence
Max. diameter of coch.	(mm)		ca. 7		6.5		7[illegible]	*v*	Good agreement
Height of cochlea	(mm)		ca. 6			ca. 6		*w*	Good agreement

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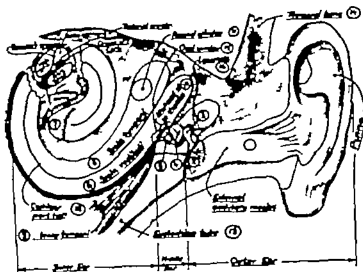


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Table II

Item	I	II	III	IV	V	VI	Dim units
	Place of measurement along cochlear partition (semiturns)						
	I	II	III (Mean of 5 specimens)	IV	V	VI	
a_1	0.1	0.20	0.29	0.34	0.38	0.42	mm
a	0.08	0.16	0.21	0.25	0.28	0.3	mm
b	0.2	0.35	0.43	0.5	0.55	0.6	mm
c	36	53	63	70	72	75	μ
d	43	67	72	79	88	109	μ
e	50	65	73	83	90	105	μ
g	0.21	0.2	0.225	0.245	0.24	0.24	mm
h	26	20	15	12	11	10	μ
i	15	27	32	35	37	38	μ
j	4.7	2.8	4	2.8	3.3	3	μ
k	7.2	7.5	7.7	7.2	8.0	8.0	μ
l	25	28	32	36	38	40	μ
m	0.95	0.8	0.6	0.5	0.47	0.45	μ
o	105	95	90	88	85	82	μ
p	60	65	60	70	75	80	μ
q	2.6	2.7	2.7	2.8	2.7	2.6	μ
r	2.8	3.0	2.9	3.1	3.0	2.9	μ
s	2.9	2.[illegible]	2.1	2.0	1.9	1.8	μ
t	40	60	75	85	90	100	μ
u	4.9	4.0	3.3	[illegible].7	2.4	2.0	μ
v	165	130	115	105	95	90	μ
w	8.0	6.0	5.2	3.6	3.3	3.0	μ
x			0.3				

For explanation of items, cf Fig. 5

connected (musculus tensor tympani) and another one (musculus stapedius) to the top of the stirrup The latter is the smallest muscle of the body Their effect has been studied extensively (10 28, 29 et al)

Békésy (23) has observed that the stapes may vibrate in two different modes If the ear is exposed to weak and medium sound intensity the footplate of the stapes rotates about a vertical axis which runs approximately perpendicular to the handle of the incus, and through the ligament which holds the footplate at the side of the other ossicles The footplate then acts like a flap as mentioned earlier

At elevated sound intensities the axis of rotation turns 90 and coincides with the longitudinal axis of the footplate. The volume of fluid pressed in and out of the scala is then reduced as the volume forced inwards on one side of the axis of rotation is compensated to a great extent by the volume escaping on the other side of the axis of rotation in the other direction. For this mechanism which protects the ear no explanation is found in literature

On the basis of the investigations carried out, the physical basis seems, however obvious. As the musculus stapedius contracts if the ear is exposed to an excessive sound, this will influence the mechanical behaviour of the system The tendon of the musculus stapedius runs parallel to the longitudinal axis through the footplate. Therefore a contraction of this muscle will reduce the degree of freedom of the oscillating footplate accordingly and a vibration about a new axis will result.

Whereas the drum is a form of energy converter (pneumatic into mechanical energy) the chain from the drum over the ossicles to the perilymph does not only transform mechanical to hydraulic energy but it also may be termed impedance converter matching the characteristics of the liquid in the inner ear to that of the air in the outer ear

(3) *Inner Ear* The inner ear comprises the extremely involved system of the cochlea which is so named due to its resemblance to

a snail shell. In terms of evolution it is the youngest part of the labyrinthine system, and is found in mammals only (30, 31).

Next to the cochlea in the labyrinth, or stato-acoustic organ, is the vestibular apparatus from which the cochlea has developed. This system responds to motion and position and does not concern us directly here.

The inner ear consists of a very complicated system of parts, many of which are minute (cf. Table II and Fig. 5). This part of the acoustic instrument has earned the reputation of being the most complicated organ of the human body.

As the exact design and performance of this part of the hearing instrument is the subject of discussion, a somewhat more detailed description will be appropriate.

The cochlea consists of the three ducts which are (i) the scala vestibuli, (ii) the scala tympani, and between these two (iii) the ductus cochlearis or scala media (cf. inset at upper left corner of Fig. 1 showing there a cross section of the ducts, and Fig. 5). Scala vestibuli and scala tympani are interconnected at the helicotrema at the apex of the cochlea, where the ductus cochlearis ends. Scala vestibuli and scala tympani are filled with perilymph, which is similar to extracellular fluid (K = 4.8 meq./liter, Na = 153.7 meq./liter, Cl = 120.5 meq./liter). The endolymph in the scala media is very similar to the electrolyte concentration of intracellular fluid (K = 137.7 meq./liter, Na = 15.2 meq./liter, Cl = 107.7 meq./liter (32)).

There is a steady d.c. potential in the scala media of about 50 to 100 mV, which is positive with respect to the scalae vestibuli and tympani. The tunnel and other spaces within the organ of Corti are filled with perilymph (32). Some authors call it cortilymph (33). Reissner's membrane and the membrana reticularis are impermeable to sodium and potassium ions, the basilar membrane is, however, permeable. The potential difference from cells of the organ of Corti to the perilymph is 80 to 130 mV. This d.c. potential, however, is not the source of the cochlear microphonic which is discussed later, according to recent studies (34, et al.). The fact that the spaces in the organ of Corti are filled with perilymph, enables the un-myelinated nerve fibers crossing them, to conduct impulses (32, 35).

The scala tympani and the scala vestibuli are partly separated by a bony shelf (lamina spiralis ossea). The gap between this lamina and the spiral ligament is bridged by the basilar membrane. As the distance between the lamina spiralis ossea and the spiral ligament grows from the base of the cochlea, i.e. near to the stapes, to the apex at the helicotrema, the width of the basilar membrane grows progressively too. The basilar membrane is reinforced internally by many fibers. Békésy (36) has reported isotropic mechanical properties, Iurato (37) found anisotropic structure with most fibers running transversely.

The bony shelf carries the limbus spiralis, at the upper side of which are the teeth of Huschke (cf. Fig. 4). There are about 7 000 of them along the cochlear partition. To their upper side the membrana tectoria is attached. This membrane is internally reinforced by many fine fibrils (38–40), which run practically parallel to each other, and at an angle of about 20 to the transversal direction of the cochlear partition (cf. Fig. 2). There is practically no longitudinal interlacing of these reinforcing fibers. These fibers can be clearly seen in Figs. 7 and 9. Due to this internal reinforcement the tectorial membrane possesses considerable anisotropic mechanical properties.

The lower side of the basilar membrane carries a layer of connective tissue and a spiral blood vessel, the upper side carries the organ of Corti.

The organ of Corti (cf. Fig. 5) consists of a row of inner and a row of outer pillars which are placed on the basilar membrane at some distance from each other. The rows of pillars meet at their top. They are internally reinforced by fibers. At their top the reticular lamina is attached, which is also reinforced

Table II

Item	Place of measurement along cochlear partition (semiturns) I	II (Mean of 5 specimens)	III	IV	V	VI	Dim. units
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j	4 7	2.8	4	2.8	3.3	3	μ
k	7 2	7 5	7 7	7.2	8 0	8.0	μ
l	25	28	32	36	38	40	μ
m	0.95	0 8	0 6	0.5	0.47	0 45	μ
o	105	95	90	88	85	8[illegible]	μ
p	60	65	60	70	75	80	μ
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r	2.8	3 0	2.9	3 1	3 0	[illegible]9	μ
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The inner ear consists of a very complicated system of parts, many of which are minute (cf. Table II and Fig. 5) This part of the acoustic instrument has earned the reputation of being the most complicated organ of the human body

As the exact design and performance of this part of the hearing instrument is the subject of discussion, a somewhat more detailed description will be appropriate.

The cochlea consists of the three ducts which are (i) the scala vestibuli, (ii) the scala tympani, and between these two (iii) the ductus cochlearis or scala media (cf inset at upper left corner of Fig. 1 showing there a cross section of the ducts, and Fig. 5). Scala vestibuli and scala tympani are interconnected at the helicotrema at the apex of the cochlea, where the ductus cochlearis ends. Scala vestibuli and scala tympani are filled with perilymph, which is similar to extracellular fluid (K ~4 8 meq / liter Na ~153 7 meq /liter Cl ~120.5 meq /liter). The endolymph in the scala media is very similar to the electrolyte concentration of intracellular fluid (K ~137 7 meq /liter Na ~15.2 meq /liter Cl ~107 7 meq /liter (3)).

There is a steady d c. potential in the scala media of about 50 to 100 mV which is positive with respect to the scalae vestibuli and tympani. The tunnel and other spaces within the organ of Corti are filled with perilymph (32). Some authors call it cortilymph (33) Reissner's membrane and the membrana reticularis are impermeable to sodium and potassium ions the *basilar* membrane is, however permeable. The potential difference from cells of the organ of Corti to the perilymph is 80 to 130 mV This d c. potential, however is not the source of the cochlear microphonic which is discussed later according to recent studies (34 et al.). The fact that the spaces in the organ of Corti are filled with perilymph, enables the un-myelinated nerve fibers crossing them, to conduct impulses (32, 35)

The scala tympani and the scala vestibuli are partly separated by a bony shelf (lamina spiralis ossea). The gap between this lamina and the spiral ligament is bridged by the basilar membrane As the distance between the lamina spiralis ossea and the spiral ligament grows from the base of the cochlea, i.e. near to the stapes, to the apex at the helicotrema, the width of the basilar membrane grows progressively too The basilar membrane is reinforced internally by many fibers. Békésy (36) has reported isotropic mechanical properties, Iurato (37) found anisotropic structure with most fibers running transversely

The bony shelf carries the limbus *spiralis*, at the upper side of which are the teeth of Huschke (cf. Fig. 2). There are about 7 000 of them along the cochlear partition. To their upper side the membrana tectoria is attached. This membrane is internally reinforced by many fine fibrils (38–40) which run practically parallel to each other and at an angle of about 20 to the transversal direction of the cochlear partition (cf. Fig. 2) There is practically no longitudinal interlacing of these reinforcing fibers. These fibers can be clearly seen in Figs. 7 and 9 Due to this internal reinforcement the tectorial membrane possesses considerable anisotropic mechanical properties.

The lower side of the basilar membrane carries a layer of connective tissue and a spiral blood vessel, the upper side carries the organ of Corti.

The organ of Corti (cf. Fig. 5) consists of a row of inner and a row of outer pillars which are placed on the basilar membrane at some distance from each other The rows of pillars meet at their top They are internally reinforced by fibers. At their top the reticular lamina is attached, which is also reinforced

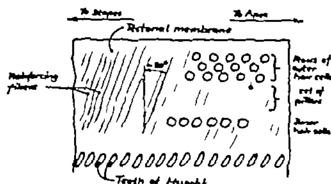


Fig 2 Top view (so-called surface preparation) of the tectorial membrane. The rows of the outer hair cells and the inner row can be seen. Further the *feet of the rows of outer and of inner pillars* have been shown in this schematic drawing. The angle of the reinforcing fibers in the tectorial membrane is about 20° In the bottom of the illustration the row of teeth of Huschke is shown. The tectorial membrane is attached to the top of these teeth.

a strong platelike structure. The inner rim of the reticular lamina is attached to the border cells, which in turn rest on the basilar membrane the outer edge is connected to the cells of Hensen which also rest on the basilar membrane

In the inner portion of the reticular lamina is mounted a row of hair cells (transducers) i.e. the upper rim of the transducers is attached to the reticular lamina The lower side of each transducer rests on a phalangeal cell, which is internally reinforced by fibers The phalangeal cells are placed on the basilar membrane.

The top of the transducer is closed by a cuticular plate This cuticular plate carries a set of hairs due to which these cells are called hair cells

The outer portion of the reticular lamina carries normally *three rows of outer hair cells,* the design and mounting of which corresponds *to that of the* inner hair cells There are about 3 500 inner and about 20 000 outer hair cells. The outer rows of hair cells are off set against each other (cf Fig 2) in a very regular way as may be seen in surface preparations of the organ of Corti (41 42 et al) Whether the hair cells are connected to the tectorial membrane or not is still disputed (41–44 et al)

According to present views (45–53) nerve fibers terminate in tulip-like endings at the *lower portion of the inner hair cells* The outer hair cells show three types of nerve endings, again arranged *at the lower portion of the hair* cells.

The efferent *nerve fiber bundle contains* about 500 fibers. There are further about *25 000 afferent fibers (45) The efferent fibers* are mostly collected in bundles, like in a switchboard at the spiral tunnel bundle *from* where they reach the outer hair cells, and again at the inner spiral bundle from where they pass between the foot plates of the inner row of pillars (rods of Corti) to join the spiral tunnel bundle (38 54) From the transducers across the tunnel and along the bundles until the fibers reach the habenula perforata, the nerve fibers are not myelinated At the latter place, i.e at the outer edge of the bony shelf the myelin sheet starts and covers the nerves on their further path

A little further on, the nerve fibers reach the spiral ganglion which contains about 25 000 bipolar cells. No synaptic connections are recognized there. The cell bodies in this long spiral ganglion are more densely grouped towards the base (900 to 1 100 per mm) than towards the apex (about 500 per mm) The transition is gradual (55)

From the spiral ganglion the auditory nerve leaves the cochlea through the modiolus (internal auditory meatus) (cf Fig 3) The fibers in the auditory nerve are *arranged in an orderly* way and twist like the strands of a rope.

A *detailed description of the inner* ear may be obtained from the references referred to in *this chapter* and later on. The physical properties of the inner ear will be described when discussing the cybernetic model of the theory of hearing proposed later on

(4) *Auditory Pathway and Cortex* Although the higher neural pathways and the auditory cortex do not belong to the ear system within the scope of this paper a brief description will be given as reference is made to it later on.

A schematic

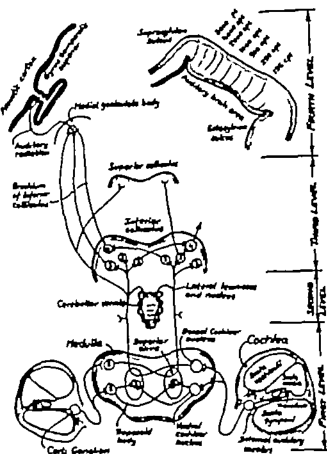


Fig. 3. General arrangement of the auditory pathway between the cochlea and the auditory cortex of the brain. On the right the different levels have been shown. Further more numbers from 1 to 4 inserted inside the synapses show the order of neurons.

From the second level onwards only the connections to the left hemisphere of the brain have been shown, whereas in the upper right corner the distribution of frequencies on the auditory brain area is indicated.

Attention is drawn to the cross connections between both ears and both brain hemispheres at the medulla and at the inferior colliculus. The pathways have been shown in very simplified way to maintain lucidity

tory pathways and of the auditory cortex are shown in Fig. 3

This system may be divided into a first level which comprises the nuclei in the lower brain stem (superior olive trapezoid body and lateral lemniscus), the second level which covers the cerebellum, the third one with the inferior colliculus at the upper pontine level and finally the fourth level from the medial geniculate body to the cortex.

The order of the neurons has been indicated in Fig. 3 according to the mostly adopted system (56).

It is seen that both sides are interconnected at various levels, which is important when considering certain observations on hearing. It is also seen that the frequencies are arranged in an orderly way on the cortex (57) however such evaluations have practically exclusively been done on animals cortex. There is, however no reason why the human cortex should not be arranged in a similar way

Regarding the electrical phenomena of the hearing instrument and of higher centers, one will have to start with the aural or cochlear microphonics. If electrodes are placed on the round window and at some place of head or neck tissue, an electrical potential is observed which is generated within the cochlea and which represents the sound pressure change of an acoustic stimulus very well. This electric potential remains nearly proportional to the

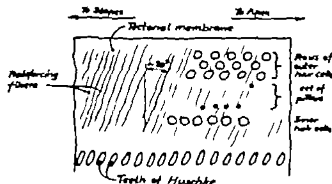


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A schematic arrangement of the neural audi

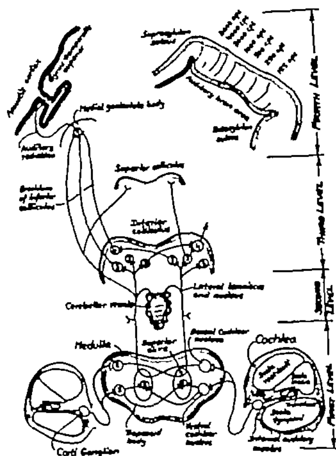


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sound pressure over a considerable pressure range (27) It also appears due to a bone con ducted sound if drum, malleus, and incus have been removed surgically and after degeneration of the auditory nerve upon transsection at the internal auditory meatus

The polarity of the cochlear microphonic at the round window is opposite that at the oval window as if the generator would be located on and perpendicular to the cochlear partition. The origin of the cochlear microphonic is still uncertain and has been attributed nearly to any structure within the cochlea (27 et al.) How ever the fact that congenitally deaf animals which have no organ of Corti but a normal basilar membrane and Reissner's membrane, produce no cochlear microphonics, indicate that the generators may be located within the organ of Corti This, and the polarity accounts for the generally accepted hypothesis to attri *bute the cochlear microphonic to the hair cells* (transducers)

The electrical potential in the eight nerve (auditory nerve) follows the stimulating frequency only up to about 1 200 cps The reason *is that the activity of each of the about 30 000* nerve fibers in the auditory nerve follows the all-or none character of nerve action potentials (58–60) This means that a stimulus above a certain threshold will trigger an action poten tial of relatively constant amplitude (disregarding, e g. reduced amplitudes for repetitive re sponse (60), or if the frequency is such that each subsequent action potential falls into the latter part of the refractory period (59)) The all-or none rule is to a certain extent a misnomer because stimuli below threshold cause effects at synaptic connections (e g. facilitation) and at ganglions As synapses are *normally* served by several connections, the number as well as the intensity and/or time pattern of the in-coming signals is of importance (61–63) Synaptic connections work like one-way valves, *transmitting stimuli only* in one direction

The absolute refractory period, during which no second action potential can be induced, determines a certain *maximum* frequency of the action potentials in a nerve fiber which cannot be exceeded. Therefore, at higher acoustic frequencies *the individual nerve fiber* cannot respond to every sound wave. However the tendency towards nearly synchronous re sponse from a group of fibers is very strong (61) From the third neuron onwards synchronization is less *precise due to variations in* synaptic delay

If the ear is exposed to a steady tone, the discharge frequency of a second-order neuron falls off to fifty percent or even less of the original rate, *within a short time. After* adaptation a steady discharge rate is maintained by most neurons.

From these few features discussed here it is seen that the original frequency is not transmitted along the orderly arranged pathways to the auditory cortex, which also shows regular frequency arrangement, but it is evident that some coding is involved, which has been studied extensively (64–71 et al.)

A further feature which deserves attention is that first-order neurons (34 72–75) and second order neurons (61 76) are frequency *selective At a certain frequency* to which a certain neuron seems to be tuned, less acoustic energy is required to set up impulses. At rather low sound intensities tuning is very sharp. If the intensity is raised the response extends more to the low frequency side (61)

Due to the enormous technical difficulties when trying to single out responses from one of many *thousands of elements crammed into* minute space, and considering superimposed fields an exact answer to the many questions has not been obtained so far Even in the case of the cochlear potential, recording of which is simpler Békésy (77) has cautioned not to interprete findings too literally

The above very brief description gives *only* a rather general idea *of the very involved* mechanism features and problems of neural performance to which those of synaptic mechanisms have to be added. In addition to the references cited above, *standard handbooks* (54 58 59 78–82) may be helpful.

Experimental Procedure

The investigations were carried out on the ear system of the water buffalo (*Anoa bubalis*) as found in Pakistan. The reasons for this selec tion were

(1) The dimensions of the parts to be investigated are so small in ordinary laboratory animals, like white rat, cat, rabbit, and guinea pig, that it seemed desirable to choose the ear system of the biggest animal conveniently available for investigation.

(2) The treatment of the parts to be investigated involves the danger of considerable distortion or even damage to the parts after slaughter of the animal, during fixation and further processing. It was assumed that the bigger and, therefore, more rigid system of the ear of the water buffalo would develop less distortion and retain its normal shape better than the smaller ones.

(3) Large numbers of samples had to be investigated, firstly to develop a suitable technique and secondly to increase the accuracy of the results. Therefore, the availability of specimen in sufficient number had to be assured.

(4) The ear system of water buffalo was available immediately after slaughter of the animal. This was necessary because otherwise post-mortem changes might have taken place distorting the specimen and thus affecting the results of investigations in uncertain manner

(5) It was expected that the dimensions of inner ear of the water buffalo are similar to those of the human ear and, therefore, would permit convenient comparison of data.

To verify the point (5) above, a survey of the outer and of the middle ear system and of the general dimensions of the inner ear of the water buffalo was made before investigating the details of the cochlear partition. This sur vey also permitted, later the assessment of shrinkage of test pieces during processing. Re sults of these measurements are given in Table I. For convenient comparison the dimensions of the human ear have been entered.

Tissue technique

The description hereunder is restricted to few important aspects only

The time between slaughter of the animal and placing of tissue in fixation solution was reduced to about 15 min. A minimum delay is of utmost importance to avoid post-mortem de terioration (83) Before fixation the volume of bone was reduced as much as possible to enhance fixation and decalcification speed.

The fenestra rotunda and the footplate of the stapes were opened under fixation solution *very carefully* avoiding any pressure on the perilymph within the scalae. Then the scalae were flushed repeatedly restricting the column of the flushing solution to 3 to 6 mm.

The fixation solution suggested by Wittmaack (84) was modified to contain double distilled water = 185 ml, $K_2Cr_2O_7$ = 4.25 g, HCHO = 10 ml, CH_3COOH = 5 ml, OsO = 0 7 g. The fixation solution was mixed immediately before use, and fixation was effected in the dark to avoid formation of artificial pigments, and to reduce side effects regarding solubility of precipitated proteins. Fixation time was 48 hours, changing the first solution after 24 hours. The temperature was kept at 25 C.

The test piece was then washed under running water for 24 hours.

Decalcification was effected with 300 ml of 7.5 % HNO_3, the solution was replaced daily by a fresh one, and decalcification lasted 8 days. The tissue was suspended in the upper part of the solution

For exact and convenient embedding of specimen a plane was cut on the bone through the vestibular apparatus and about 5 mm from the stapes.

Before dehydration the specimen was placed in 300 ml of 5 % Na_2SO_4 for 24 hours, and then washed under running water for the same period

Dehydration was started with a 30 % alcohol solution containing 1 % phosphotungstic acid *to forestall later collection of the previously* hydrated fiber-colloids during the concentrated alcohol steps (85) The 30 % and 50 % steps of 6 hours each were followed by 60 70 80 90 and 96 % alcohol water mixtures and two steps of absolute alcohol each step lasting 24 hours. Dehydration was carried out in dark.

The tissue was cleared in two changes of pure 100 % benzene each lasting 24 hours, and this was followed by 48 hours of infiltration *in benzene which was saturated with* paraffin of 58 C melting point. Two changes of molten paraffin (58 C melting point) followed each of 24 hours, and using about 250 ml pure paraffin each time

The test piece was placed on its orientation plane in a cylindrical aluminium container in the last bath, and then the container was placed in cold water To ensure uniform solidification of the paraffin its upper layer was kept molten until the lower part had solidified.

A rotary microtome was employed for sectioning. Because of the distance of pillars and transducers sections were made 10 micron thick. The samples were cut from the apex to the base of the cochlea in this way the delicate structures of the organ of Corti are supported by the basilar membrane Figs. 6–11 show an *increasing compression* of the architectural structure within the organ of Corti from stapes to apex, and which gets less compensated in the same direction by *natural elasticity of the* structures during flattening of paraffin sections during mounting

To be able to produce *the considerable number* of high quality sections required without the lengthy procedure of celloidin embedding, the somewhat complicated cellulose tape method suggested by Palmgren (86) was simplified considerably *(87) Such cellulose tape* sections behave like celloidin sections and are therefore very little compressed by the cutting force of the microtome knife They do, therefore, not show the interesting effect of standard paraffin sections, which was discussed above, but they conceal this property of gradually varying elasticity along the cochlear partition. *However* this change of elasticity of structures is of great interest and will be dealt with in detail in the text For this reason an adequate number of normal paraffin sections was made.

For staining either molybdenum hematoxylin *(83 88) or molybdenum hematoxylin* combined with picro fuchsin was used

Physical measurements

*All parts were measured with an ocular micro*meter employing magnifications of 125 400 and 900 times, according to size of the part to be measured Oil immersion and a suitable illuminator and filter sets to obtain near monochromatic illumination of short wavelength was used for the highest magnification.

To obtain a clear and complete picture of the interaction of the different parts of the system and of their dimensions as a function of the distance of the place of measurement from the stapes, investigations were made at a distance of 10 19 25 30 33 and 36 mm from *the stapes If proper* measurement of length of individual structures seemed to be difficult due to bending of the part to be measured in some test specimen this was overcome by reconstructing the architectural structure in precise drawings with the aid of a to-scale photograph of the section and making of a model of soft copper wire which was subsequently straightened

To compensate for physiological deviations in size of individual ear systems, the values for each ear were tabulated separately and the changes in dimensions along the cochlear partition were ascertained individually for each test piece.

Thirty left ears of water buffaloes of about 6 to 9 years age have been studied. The inclusion of old animals is important because it is believed by some authors (89–92) that the tectorial membrane is only attached to the hairs of the transducers during fetal life

Results of Observations

The results of the measurements of the outer and middle ear system and of the general dimensions of the inner ear are given in Table I The places of measurement are indicated in Fig. 4

As may be seen the main dimensions of the inner ear of *Anoa bubalis* and of man are very similar Some of the dimensions of the outer ear differ These differences suggest an enhanced transmission of lower frequencies by the outer ear of the water buffalo which is of no great importance in connection with the main part of the investigation.

The main part of the investigation, concerning the inner ear has resulted in a clear picture of the interconnection and action of this part of the system

The dimensions of the parts of the cochlear partition are given in Table II In Fig. 5 the places of measurement have been entered, as well as numbers of parts used in the text of Fig. 5 and in later photomicrographs of semi turns of the cochlea

Before discussing dimensional results it seems correct to mention some general observations first.

So far there has been no agreement about any possible interaction between the membrana tectoria and the organ of Corti. Whereas it was generally accepted that the membrana tectoria is attached to the organ of Corti during fetal life (31 41 89-92) the position after birth was not defined

Any detachment of this membrane from the other portion of the cochlear partition must change the physical properties of the inner ear considerably Such change has not been reported in literature. On the contrary it has been established that the animal and human fetus responds to acoustic stimulation in the form of reflexes. Further electric responses can be recorded at fetal inner ears (92) This proves that the inner ear is fully functioning at the time when the tectorial membrane is accepted to be attached.

This investigation shows that the tectorial membrane is a functional part of the entire system of the cochlear partition. Any detachment of this membrane from the organ of Corti is caused by inadequate tissue technique. The solution and method developed in this investigation minimizes this disadvantage and detachment occurs only near to the stapes

This means that the force of contraction due to shrinkage is greater towards the stapes This can be explained by the fact that the number of fibers is greater there and their diameter is greater near to the stapes. Therefore the total cross-section and force of contraction of fibers is increasing towards the stapes.

Due to this variation the stiffness of the membrana tectoria increases towards the stapes This is borne out by experiments in the direction of decreasing stiffness, i e towards the apex, the deformation of the membrana tectoria increases under the sectioning force of the microtome knife

In a detached membrana tectoria the considerable number of fibrils which are oriented in a particular way (cf Fig. 2) would not make much sense It would thus seem rather doubtful what purpose this structure serves, being in the way of hydraulic waves coming from the scala vestibuli through the scala media to the organ of Corti. If the tectorial membrane would not be connected to the organ of Corti it would only consume valuable energy without serving any useful purpose

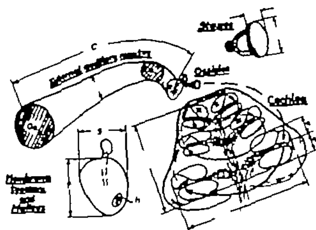


Fig. 4 Schematic drawing of the parts of the outer, middle, and inner ear indicating the parts measured and given in Table I. Also semiturns I–VI are shown, where detailed dimensions of all essential parts of the cochlear partition have been obtained, which are contained in Table II.

Furthermore, the tectorial membrane is formed rather early by the epithelial cells, during the development of the human or animal body. This indicates that it is an essential part.

The orientation of the fibrils in the main body of the tectorial membrane runs obliquely towards the apex of the cochlea, at an angle of about 20°. This construction which has

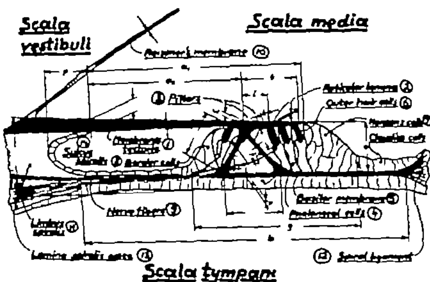


Fig. 5 Schematic section of the organ of Corti indicating places of measurement. This section shows the third semiturn in scale of 200:1. The gap between the membrana tectoria (1) and the reticular lamina (2) is about 1 to 2 micron wide.

(1) Membrana tectoria (2) Reticular lamina
(3) Pillars (4) Phalangeal cells
(5) Basilar membrane (6) Outer hair cells
(7) Hensen cells (8) Border cells
(9) Nerve fibers (10) Reissner' membrane
(11) Limbus spiralis (12) Spiral ligament
(13) Lamina spiralis ossea (14) Sulcus spiralis

From the dimensions of the parts, which have been determined for six places along the cochlear partition, precise reconstruction of the instrument is possible. For dimensions please refer to Table II.

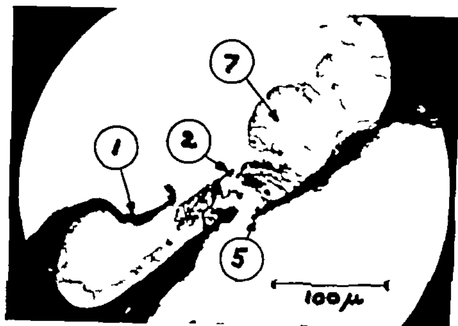


Fig 6 First semiturn of 10-year old buffalo. Magnification ~66 times. Membrana tectoria (1) is torn lose from the organ of Corti. The reticular lamina (2) is also torn. Attention is invited to the cells of Hensen and of Claudius (7), which are far more bulky than the organ of Corti. The thickness of the basilar membrane (5) is not uniform.

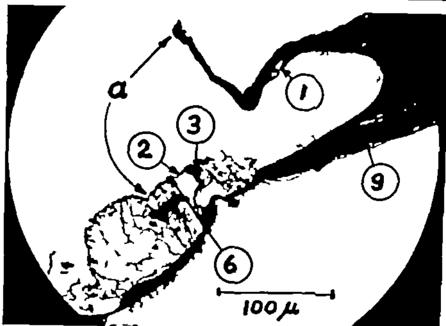


Fig 7 Second semiturn of same test piece. The reinforcing fibers within the tectorial membrane are clearly seen (1). Similarly these fibers are to be seen in the reticular lamina (2) and in the head of the pillars (3). Also hairs of outer transducer rows (6) are visible, as well as nerve fibers (9). It is very interesting to note how nicely the end of the tectoria (1) (upper arrow of (a)) fits into the corresponding recess at the outer end of the reticular lamina (lower arrow of (a)). This leaves no doubt about the attachment of the tectorial membrane to the organ of Corti along its entire length.

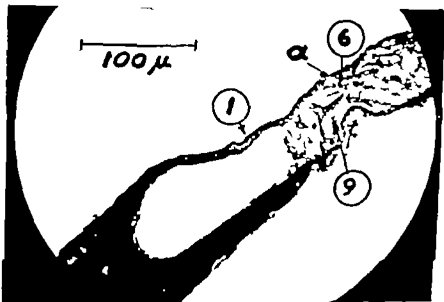


Fig 8 Third semiturn of same specimen. The gap below the tectorial membrane is very well seen at arrow (a). This gap is bridged by the hairs at the upper end of the transducers (6). A nerve fiber with cell body (9) can be seen crossing the tunnel

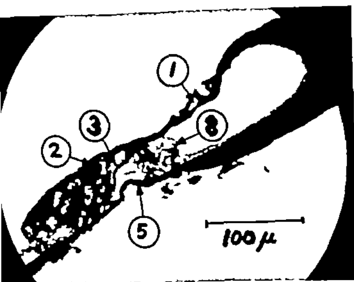


Fig 9 Fourth semiturn. Attention is drawn to the thin reinforcing fibers in the tectoria (1), to the gap bove the reticular lamina (2), the decreased thickness of the basilar membrane (5), and to the bulge at (8). The reason for the bulge is compression during processing of the specimen.

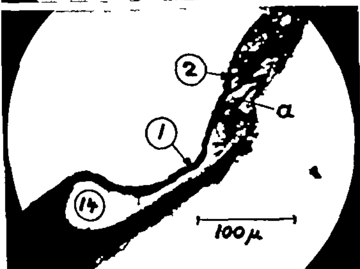


Fig 10 Fifth semiturn. The increase in length f the membrana tectoria (1) compared to Fig. 6 is striking. The membrana reticularis is to be seen at (2), and very clearly the gap between it and the tectoria where the reticularis has bulged inwards (*a*). The length of the sulcus spiralis should be compared with Fig. 6.

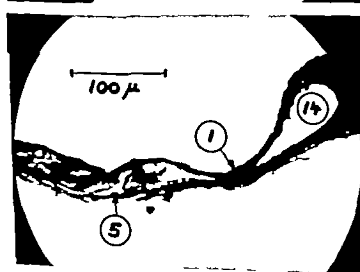


Fig. 11 Sixth semiturn. Here the membrana tectoria (1), as well as the basilar membrane (5) have become rather thin, as compared to the first semiturn. The sulcus spiralis should be noted, it indicates the span of the tectoria.

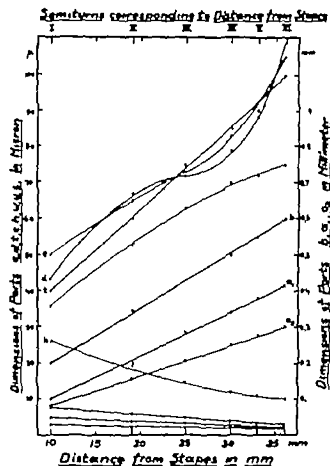


Fig 12 Dimensional changes of the most important parts of the architectural structures of the cochlear partition as a function of the distance of the place of measurement from the stapes.

Roman numerals on the abscissa indicate the distance of the semiturn from the stapes. On the ordinate dimensions are shown in millimeter or in micron according to the size of the part and in accordance with *Table II*

The continuous curves demonstrate the gradual change of dimensions between the stapes and the apex of the cochlea

been noticed by several workers (e g. 37 38 40) but which *has not* been incorporated in theories of hearing has to be considered because it results in anisotropic mechanical properties of the membrane. Such directional elasticity has been observed by Békésy (96) in 1947 but it has not been incorporated in any of the current theories of hearing.

Further experiments show that the dimensions of the membrana tectoria change gradually as a function of the distance of the place of measurement from the stapes The thickness of this membrane decreases from the stapes to the apex, and at the same time the width of the membrane increases in the same direction This can very well be seen in Table II and in Figs 6 to 11 and attention is also invited to Fig. 12 which gives the dimensional changes of the most important parts in graphs.

Due to a rather limited amount of non-orientated (interlacing) fibers (37 38 40) the membrana tectoria possesses considerable anisotropic elasticity as already observed by Békésy (loc cit.) and therefore, can be said to possess the property of an oscillator It can thus be concluded that due to small coupling to adjacent segments *sharp tuning will result.* This means that the natural frequency of the membrana tectoria *will change gradually from* a high value near the stapes to a low one at the apex (helicotrema) and that only a narrow segment will oscillate in resonance with the *frequency of the force* and also at the places of the membrana tectoria corresponding to *higher harmonics* if the sound intensity is of adequate level A detailed discussion will follow later

The length of the pillars (rods of Corti) and *at the same time their angle of inclination* increases progressively from the stapes towards the *helicotrema As their diameter remains* practically constant this features will lower the *natural frequency of the system* towards the apex

The *plate* (reticular lamina) attached to the upper side of the pillars is thicker near the stapes, at the place of reception of the high frequencies, than at the helicotrema. As the *natural frequency* of a plate goes up if its thickness is increased this increase of thickness of the reticular lamina will also raise the natural frequency of the system towards the stapes.

The span of the reticular lamina (cf *l* in Fig. 5 and in Table II) from the point where the pillars meet, outwards, increases as one moves towards the apex If the span of an oscillating plate is raised this will reduce the natural frequency and in the same manner the natural frequency of the system will be lowered towards the apex.

It has been known for at least one and a half century that the span of the basilar membrane increases considerably from the stapes towards the helicotrema. However data for variation of width do not show good agreement and vary from about 1 in 6.26 (97) to about 1 in 12.5 (25). The variation for the water buffalo is about 1 in 12 (Table I) which is in good agreement with most findings concerning the human ear.

The experiments show in addition, that not only the width increases from stapes to apex, but that at the same time the thickness of the basilar membrane decreases. This is a strong argument against any theory which considers the basilar membrane as resonator, as the mass per unit length decreases.

Further the reinforcing influence of the structures of the organ of Corti on the basilar membrane diminishes as one proceeds towards the apex. This is not only confirmed by the dimensions obtained during this investigation (Table II) but by the behaviour of standard paraffin sections, which confirms this physical property. As mentioned earlier the cutting pressure of the microtome knife was directed towards the base of the inner ear. This produced an increasing compression of the architectural structure towards the apex and which was in the same direction less and less compensated by the natural elasticity of the structure. An accidental behaviour during experiments regarding this property has to be ruled out, as each one of the very many experiments produced identical results.

The decrease in stiffness discussed above is also briefly mentioned by Hawkins (38) however there is no general increase in mass from base to apex, as he believes. As uniform density of corresponding parts along the membrane may be taken for granted, it can be derived from the values shown in Table II that the mass of the organ of Corti remains practically constant along the cochlear partition.

As observed by Hawkins (loc. cit.) the length of the transducers increases from the stapes towards the apex.

Studying now Fig. 12 and starting the discussion with the lowest curve for the thickness of the reticular lamina (s) at the first outer row of transducers, it is seen that the reticular lamina is thicker towards the stapes. From Table II one obtains that the increase in thickness from 36 mm from the stapes to 10 mm from the stapes amounts to about 60%.

The thickness of the basilar membrane (u) increases uniformly from 2 micron at semiturn VI to 4.9 micron at semiturn I, i.e. nearly two and a half times.

The curves for (w) and (h) may be considered together both give the thickness of the membrana tectoria. Curve (w) shows the value above the joining place of the pillars, and (h) is the thickness at the important place where this membrane leaves the limbus spiralis, i.e. from where its free span measures. It is seen that the thickness increases continuously from the apex to the stapes. The increase for (h) from semiturn VI to I is 260%. This increase is not quite linear along the cochlear partition, as the curves show. The curve for (h) is very important in connection with determining the natural frequency of the tectorial membrane.

The graph for (a_2) illustrates the increasing span of the membrana tectoria from the place where it leaves the limbus spiralis to the place above the junction of the pillars. The increase is practically linear. The span is nearly 3.5 times greater at the sixth semiturn compared to the value at semiturn I.

The full free span of the membrana tectoria is designated with (a_1). It is seen that the increase is nearly linear and that the span at semiturn VI is 4.. times greater than at semiturn I. The free span is of utmost importance in connection with the natural frequency of this membrane. Details in this connection will be discussed in the next chapter.

The width of the basilar membrane progresses practically linearly from semiturn I to semiturn VI, as may be seen by inspecting the graph for (b). The value of 0.6 mm at semiturn VI is in agreement with the measurements of the main dimensions of the inner ear shown

in Table I If the curve for *(b)* is extrapolated to the left, good agreement for the width of the basilar membrane is obtained at the stapes with the value shown in Table I (0 05 mm)

The length of the inner pillar has been de signated as *(c)* that of the outer one as *(d)* and the distance between the feet of the pillars as *(e)* Also these dimensions change progres sively These dimensions are interesting in as much as they permit a study of the compression of the structures of the organ of Corti under the cutting force of the microtome knife, as mentioned earlier

Finally the span of the tectorial membrane from the junction of the pillars to the outer edge of the third row of transducers is shown as *(t)* Again a linear change is observed.

It is fascinating to note the very precise construction of all these parts of the inner ear This would never be so if no specific purpose would exist for this exact design of each part.

In spite of physiological deviations between individual ears, practically identical physical characteristics have been observed in the 30 test pieces investigated The lesser thickness of the tectorial membrane of some test pieces was accompanied by smaller spans, resulting in similar functions of the natural frequency along the cochlear partition

Discussion and Comparison of a New Approach to Theory of Hearing with Observations and with Older Theories

The results of the experiments lead to a new approach to theory of hearing and a new interpretation of the functioning of the ear can be put forward in the form of the following cybernetic model.

The oscillations of the perilymph in the scala vestibuli (cf. Fig. 5) are imparted through the membrane of Reissner to the endolymph in the scala media. Those parts of the membrana tectoria, the natural frequency of which corresponds to the frequency of the force, pick up the vibrations, and due to a certain amount of damping and coupling, neighbouring areas will also vibrate. Assuming a pure tone, only a rather narrow segment of this membrane will respond due to its anisotropic mechanical properties, and rather sharp tuning will result.

The oscillations of the membrana tectoria are imparted to the hairs (stereocilia) of the transducers (hair cells) which are connected with it, and these stiff transmitter pins (38) act on a diaphragm (cuticular plate). This closes the upper side of the transducers. For a mechanical interpretation, the transducers may be regarded as a tube which is filled with cytoplasm and closed at the upper end by a diaphragm acting like a piston on the contents of the tube.

The upper rim of each tube (transducer) is firmly mounted to a supporting frame-plate (reticular lamina) and which rests on the pillars and, to a certain extent on the border cells, and cells of Hensen which are connected to the inner and outer edge of the plate, and on the extensions of the phalangeal cells. The pillars are in turn supported on their lower side by the basilar membrane and the cell layer covering it (connective tissue) This membrane also lends support to the outer and inner cells mentioned above.

The lower part of each transducer is supported by a reinforced structure (phalangeal cell) and this rests on the basilar membrane.

This design corresponds to the most efficient possible use of the transducers, as strain built up within them is not impeded by other cells and they are freely accessible to the electrolyte at their circumference.

The direction of the fibrils which reinforce the tectorial membrane (cf. Fig. 2) corresponds to the oblique arrangement of the transducers. Therefore, each narrow segment of the tectorial membrane acts on a corresponding segment of the transducer rows below For a mechanical model the tectorial membrane may be considered as a long row of oscillating reeds, of progressively changing thickness and span, *built like a reed-type frequency meter however in a more involved way*

The gap between the tectorial membrane and the membrana reticularis and the diaphragm closing the upper side of the transducers (*cuticular plate*) is bridged by transmitter pins (hairs of hair-cells) This gap ensures that the tectorial membrane may oscillate undisturbed at low or medium sound intensities. At high sound intensities, which cause amplitudes approaching the order of the gap's size, the design protects the delicate transducers, because then the gap commences to act like a hydraulic shock absorber

Studying the design of energy transmission

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from the tectorial membrane to the cuticular plate through the transmitter pins, it is seen that this design increases the efficiency of the system, as the corresponding area of a segment of the membrana tectoria is about twice as big as the area of the cuticular plates, as may be seen from surface preparations of the organ of Corti (41 42 cf Fig 2) The effect is, therefore, comparable to the one produced by the difference in area between drum and the footplate of stapes

The displacements of the diaphragm closing the top of the transducers, act on the practically incompressible cytoplasm which in turn transmits the pressure changes to the walls of the transducers and to the fine nerve endings there

Stretching of highly polymerized chain compounds, changes their chemical properties (98) and may well induce electrochemical processes besides affecting the inter-molecular fields of forces. Stretching of a muscle causes a signal in the nerve endings in it (e g. patellar reflex) The strain in transducer wall and nerve sections attached to it, which is caused by the displacement of the contents of the transducer causes an electrical phenomenon which leads to a transmission of a signal to the auditory areas of the brain.

Some theories, like the Helmholtz theory of hearing, stipulate membranes under tension During the investigations discussed here no tensions could be found to exist on the contrary there seems to exist some loose play in the basilar membrane towards the apex It is apparent that no parts under continuous internal stress are required to explain this theory of hearing. On the other hand, the vibrating system is immersed in a liquid the relative density (31 32) of which is nearly identical to that of the other parts of the system with the result that buoyancy counteracts gravity This is an important fact in view of the extremely delicate design of the system and this does not only eliminate continuous stress but also insures identical behaviour of the system irrespective of the position of the head

As the parts of the inner ear system are not exposed to loads when at rest the many reinforcements within the various parts may be taken to indicate that they are designed to operate under dynamic forces, which interpretation sounds reasonable if one considers that the inner ear has to process hydraulic oscillations, i.e dynamic events.

Thus, when interpreting the physical behaviour of the system as described above, the intricate design of each single part and the interconnection of all parts become obvious. The objection raised against most of the theories of hearing proposed until now is that they do not consider the complicated design of the cochlear partition, which is now utilized to explain the function of the ear in a better way and seems to be confirmed by the results of this investigation. Each part of the system is required to fulfil a specific purpose and action, it is not anymore necessary to stipulate undefined analysing mechanisms as sometimes resorted to until now (99–103) and that the performance of the hearing instrument can now be well understood. (A detailed discussion of theories of hearing and of problems arising out of them will be published in a separate paper) Therefore this cybernetic model, so-called because the hearing instrument is employed to analyse and to transmit information (104–108) in which the anisotropic tectorial membrane represents a series of tuned reeds acting on segmented rows of transducers, including their suspension and all other parts, is so excellently designed by nature that it seems very difficult to suggest any improvement of the instrument

From the experimental data given in Table II the natural frequency of the tectorial membrane was calculated as a function of the distance from the stapes. For this purpose the tectorial membrane was considered like a series of bars. This approach was adopted because of the anisotropical properties observed by Békésy (96) which show a rather narrow segment of depression even under excessive load and because this property is fully confirmed by the

arrangement of the reinforcements in the membrane (loc. cit.) Under normal sound intensities a very much narrower segment will be affected than by the heavy load applied by Békésy and which permits treatment of individual sections like bars, at least to a first approximation.

The physical properties of the parts described above indicate that attention has to be focused on the membrana tectoria, although one will have to consider a number of contributing factors which will influence the natural frequencies of the structure along the cochlear partition. Such factors are the progressively decreasing moment of inertia of the membrana reticularis towards the apex, the similarly decreasing restoring force exercised by the pillars, and the cross section of the fibers reinforcing the membrana tectoria which decreases progressively towards the apex.

Whereas the first factors do not appear to change the characteristics of the oscillating system very much, the last one will have to be considered.

In view of the oblique arrangement of the reinforcing fibers within the tectoria (cf. Fig.) the measured values of the oscillating span of this membrane (a) as shown in Table II have been divided by cos 20 = 0.94

For the evaluation of the natural frequency of the tectorial membrane at each place of measurement, the following general formula has been used.

$$n_e \sim c(1\ f) \qquad (1)$$

where n_e is the natural frequency, c is a constant, and f is the deflection of the bar under load. For a rectangular bar clamped at one side the deflection is:

$$f \sim P\ P\ \alpha\ E\ I \qquad (2)$$

Where P is the load, l is the length of the bar, α is a constant, E is Young's modulus, and I is the moment of inertia given by the relation:

$$I = b\ h^3\ 12 \qquad (3)$$

where b is the width of the bar, h is the height.

Inserting equation (3) in equation (2) and then in equation (1) the natural frequency for a square bar ($b = h$) is:

$$n_e \sim C_I\ h^2/l^3 \qquad (4)$$

where C_I is a constant, h is the height of the oscillating bar and l is its length.

A somewhat different formula is used for calculating the natural frequency of tuning forks (109):

$$n_e \sim C_{II}\ h/l^2 \qquad (5),$$

where C is a constant, which is similar to formulas for round bars used in engineering handbooks (110)

The subsequent calculations are based on above formulas (4), and (5) and the results are shown in Table III and in Fig. 13 where also the values observed for the human ear have been entered to permit comparison. A discussion of the curves will follow later after discussion of the correction factor needed to allow for the change in reinforcements within the tectorial membrane.

The dimensions used in the calculations are as follows (cf. Table II) according to the distance of the place of measurement from the stapes (values are stated in micron):

Semiturn	I	II	III	IV	V	VI
h (μ)	26	20	15	12	11	10
a_1 (μ)	100	200	290	340	380	420
l (μ)	106	212	308	362	404	447

The thickness is h and a the free span of the membrana tectoria vertical to the direction from stapes to apex, and $l = a_1/\cos 20$ as mentioned earlier.

The constants have been chosen in such a way as to produce a natural frequency of 40 cps for semiturn VI, i.e. about 36 mm from the stapes.

The following Table III shows the natural frequency of the tectorial membrane along the cochlear partition as calculated by formulas (4) and (5)

from the tectorial membrane to the cuticular plate through the transmitter pins, it is seen that this design increases the efficiency of the system as the corresponding area of a segment of the membrana tectoria is about twice as big as the area of the cuticular plates, as may be seen from surface preparations of the organ of Corti (41 42 cf Fig 2) The effect is, therefore comparable to the one produced by the difference in area between drum and the footplate of stapes

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As the parts of the inner ear system are not exposed to loads when at rest the many reinforcements within the various parts may be taken to indicate that they are designed to operate under dynamic forces, which interpretation sounds reasonable if one considers that the inner ear has to process hydraulic oscillations, i e dynamic events

Thus, when interpreting the physical behaviour of the system as described above the intricate design of each single part and the interconnection of all parts become obvious. The objection raised against most of the theories of hearing proposed until now is that they do not consider the complicated design of the cochlear partition, which is now utilized to explain the function of the ear in a better way and seems to be confirmed by the results of *this investigation* Each *part* of the system is required to fulfil a specific purpose and action it is not anymore necessary to stipulate undefined analysing mechanisms as sometimes resorted to until now (99–103) and that the *performance of the hearing instrument* can now be well understood (A detailed discussion of theories of hearing and of problems arising out of them will be published in a separate paper) Therefore, this cybernetic model, so-*called because the hearing instrument* is em-*ployed to analyse and to transmit information* (104–108) in which the anisotropic tectorial membrane represents a series of tuned reeds acting on segmented rows of transducers, in-*cluding their suspension and* all other parts, is so excellently designed by nature that it seems very difficult to suggest any improvement of the instrument.

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stapes. It is seen that the values agree very well with the observations on the human ear. This was to be expected from the similarity obtained from the first part of the investigation, and shown in Table I. It is seen that not only the design of the outer ear of *Anoa bubalis* favours selection of lower frequencies, due to the length of the outer acoustic meatus, but that also the design of the membrana tectoria supports discrimination of lower frequencies.

The interlinkage of the parts of the architectural structures of the cochlear partition as well as their physical properties does not only permit a reasonable explanation of the mode of functioning of the system but also the values calculated from dimensions obtained from the experiments are in agreement with observations.

The result of the calculation is of great interest in connection with an observation of Békésy (111). When studying the shift of the maximum of the envelope of the displacement of the cochlear partition according to a given ratio of frequency change, Békésy has observed that the mechanical resolving power of the cochlea of the cow favours the lower frequencies.

Different theories of hearing available so far are to a considerable extent *ad hoc* relative to few phenomena only. Therefore, it will be interesting to see that this theory of hearing does not have this disadvantage, and is able to explain most of the essential observations about the behaviour of the ear. In the subsequent pages these physical problems will be discussed in the light of this theory.

A. Shape of resonance curves observed by Békésy

Békésy (111) determined the locus of maximum displacement of the cochlear partition as a function of the frequency for various animals and found curves which were similar. However, he observed that the curves for the elephant are more pointed, a phenomenon which was not investigated further.

As the intensities of tones, which are required for making direct observations are very high and mostly above the threshold of feeling, pronounced harmonics will appear which disturb the simple resonance curve. This is quite obvious as the amplitude of the basilar membrane at sound pressure of 10^3 dyne/cm² which is about the upper limit the ear can tolerate, is about 10^{-4} cm (112). The absolute threshold of hearing has been found to cause an amplitude of about 10^{-11} cm (25). In view of the experimental difficulties the accuracy of observation will improve with the size of the investigated object and it is safe to assume that the pointed curve observed by Békésy in the inner ear of the elephant represents the true performance of the cochlear partition to a greater extent than what one would believe to observe in the smaller specimens.

The pointed peak of resonance observed by Békésy in the biggest specimen supports the view deduced from this investigation, that most of the frequency analysis takes place in the inner ear, and that coupling to adjacent segments of the tectoria is small.

B. Difference in relative elasticity of the parts of the cochlear partition

Békésy did not incorporate the difference in relative elasticity observed by him (96) into a physical interpretation of the performance of the individual parts of the system but considered the cochlear partition as one part.

Békésy's observations are confirmed qualitatively and quantitatively by the observations and theory discussed here. Comparing the pronounced elasticity of the tectorial membrane with the rigid girder-like partition, it is evident that static as well as oscillatory deflections of the tectorial membrane must be very much bigger than those of the basilar membrane reinforced by the architectural structure erected on the top of it. This is shown by the following calculation for the third semiturn, where dimensions have been shown in Fig. 14.

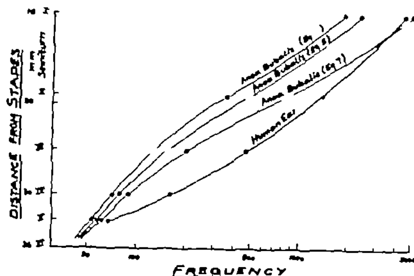


Fig 13 Diagram of natural frequencies of the tec torial membrane of water buffalo which have been calculated on the basis of data obtained during this investigation, and applying three alternative formulas.

For comparison the frequency curve for the human ear has been shown.

It is seen that the frequency curves of the buffalo agree well with the one of the human ear It is noted that the ear of water buffalo favours frequency selectivity in the lower frequency range. This property of the inner ear of the water buffalo agrees with findings during this investigation regarding the outer ear (Table I). This indicates that both sections are designed according to an identical teleological aim.

Table III
Values in cps

Semiturn	I	II	III	IV	V	VI
Formula (4)	2 330	448	157	79	57	40
Formula (5)	1 860	356	1 7	74	54	40

Reverting to formulas (1) and (2) it is seen that

$$n \approx c(a\ E\ I/P\ l^4)^{\frac{1}{2}} \qquad (6)$$

The letters used in the above equation correspond to those of formulas (1) and (2)

This equation shows that the natural frequency of an oscillating structure depends on its moment of inertia. As the cross section of the reinforcing fibers in the membrana tectoria changes with the distance from the stapes, formula (4) will have to be amended in such a way as to permit a correction of the frequencies shown in Table III when considering this factor

The axial moment of inertia depends on the mass of the homogenous body times the square of its distance from the axis, or translated to our case dealing with the cross sections of the fibers, the axial moment of inertia depends on the cross section areas multiplied by the square of their distance Here only the latter has to be considered, and the formula corrected to include this influence will be·

$$n_e \approx C_I\ h^2\ F^{\frac{1}{2}}/l^2 \qquad (7)$$

where F is the area of the reinforcing fibers. The other symbols correspond to those used in the preceding formulas. Table IV shows the natural frequencies of the tectorial membrane from semiturn I to VI as calculated and corrected for the increasing area (F) of the reinforcing fibers within this membrane as obtained from measurements.

Table IV

Semiturn	I	II	III	IV	V	VI
F (units)	4 45	3 13	1 65	1.25	1 13	1
$F^{\frac{1}{2}}$ (units)	2.10	1 77	1.28	1 12	1 06	1
n (cps)	4 890	865	201	88	60	40

The values of the natural frequencies of the tectorial membrane of the water buffalo derived from formulas (4) (5) and (7) are shown in Fig 13 according to the distance from the

stapes. It is seen that the values agree very well with the observations on the human ear. This was to be expected from the similarity obtained from the first part of the investigation, and shown in Table I. It is seen that not only the design of the outer ear of *Anoa bubalis* favours selection of lower frequencies, due to the length of the outer acoustic meatus, but that also the design of the membrana tectoria supports discrimination of lower frequencies.

The interlinkage of the parts of the architectural structures of the cochlear partition as well as their physical properties does not only permit a reasonable explanation of the mode of functioning of the system, but also the values calculated from dimensions obtained from the experiments are in agreement with observation.

The result of the calculation is of great interest in connection with an observation of Békésy (111). When studying the shift of the maximum of the envelope of the displacement of the cochlear partition according to a given ratio of frequency change, Békésy has observed that the mechanical resolving power of the cochlea of the cow favours the lower frequencies.

Different theories of hearing available so far are to a considerable extent *ad hoc* relative to few phenomena only. Therefore, it will be interesting to see that this theory of hearing does not have this disadvantage, and is able to explain most of the essential observations about the behaviour of the ear. In the subsequent pages these physical problems will be discussed in the light of this theory.

A. Shape of resonance curves observed by Békésy

Békésy (111) determined the locus of maximum displacement of the cochlear partition as a function of the frequency for various animals and found curves which were similar. However, he observed that the curves for the elephant are more pointed, a phenomenon which was not investigated further.

As the intensities of tones, which are required for making direct observations are very high and mostly above the threshold of feeling, pronounced harmonics will appear which disturb the simple resonance curve. This is quite obvious as the amplitude of the basilar membrane at sound pressure of 10^3 dyne/cm^2 which is about the upper limit the ear can tolerate is about 10^{-4} cm (112). The absolute threshold of hearing has been found to cause an amplitude of about 10   cm (25). In view of the experimental difficulties the accuracy of observation will improve with the size of the investigated object and it is safe to assume that the pointed curve observed by Békésy in the inner ear of the elephant represents the true performance of the cochlear partition to a greater extent than what one would believe to observe in the smaller specimens.

The pointed peak of resonance observed by Békésy in the biggest specimen supports the view deduced from this investigation, that most of the frequency analysis takes place in the inner ear and that coupling to adjacent segments of the tectoria is small.

B. Difference in relative elasticity of the parts of the cochlear partition

Békésy did not incorporate the difference in relative elasticity observed by him (96) into a physical interpretation of the performance of the individual parts of the system but considered the cochlear partition as one part.

Békésy's observations are confirmed qualitatively and quantitatively by the observations and theory discussed here. Comparing the pronounced elasticity of the tectorial membrane with the rigid girder-like partition, it is evident that static as well as oscillatory deflections of the *tectorial* membrane must be very much bigger than those of the basilar membrane reinforced by the architectural structure erected on the top of it. This is shown by the following calculation for the third semiturn, where dimensions have been shown in Fig. 14.

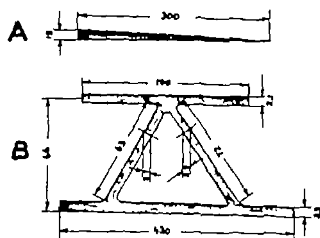


Fig 14 Schematic diagram showing the main architectural parts of the cochlear partition, with dimensions in micron for the third semiturn of the water buffalo

(A) shows the membrana tectoria s cross section, and (B) the lattice girder the base of which is the basilar membrane. On this base two rows of pillars stand which carry the membrana reticularis. The moment of inertia of the lattice girder only is nearly fifty times greater than that of the membrana tectoria It is obvious that under a static as well as under a dynamic force the deflection of the lattice girder will be much smaller than that of the tectorial membrane.

(1) Moment of Inertia of the Membrana Tectoria

$I_t = a_1 \; h^3/36 = 300 \times 15^3/36 = 28\,000$ units

(2) Moment of Inertia of the Lattice Girder

$I_{lg} = \sum I + f \; r^2$

i e the moment of inertia of the lattice girder is equivalent to the sum of the moment of inertia of the individual parts and of the area of the individual parts times the square of their distance to the axis

If the axis is taken parallel to the basilar membrane and through the center of gravity of the lattice girder a moment of inertia of about 1 000 000 units results which is about 35 times more than the moment of inertia of the tectorial membrane Actually the difference is more pronounced because the connective tissue on the lower side of the basilar membrane and other cells on top of the basilar membrane will increase the moment of inertia of the architectural structure

Considering these additional parts the difference in moment of inertia is of the same order as the difference in deflections observed by Békésy under static load (cf formula (2) regarding dependence of deflection on moment of inertia)

C. Frequency selectivity of the ear

The envelope of deflections of the basilar membrane (113 114) on which many present theories of hearing are based shows an extremely flat peak. At a sound intensity of 134 db the ratio of the height of the peak exceeding 50% of the amplitude to the length of this bulge is about 1 in 6 000! This makes it impossible to ascribe the rather delicate tuning of the ear to this envelope The peak of the basilar membrane exceeding 50% of the relative amplitude covers some octaves. This feature is one of the strongest arguments against place theories of hearing in their present form It may be suggested here that the damping of the basilar membrane is also too big to permit delicate tuning of the instrument, because one has to consider the many attached elements (cf Fig. 5) which possess internal friction.

The natural frequency of a resonating membrane is affected by its mass. However it is seen when studying cross sections of the cochlear partition that masses (cells of Claudius, cells of Hensen, border cells, connective tissue) are arranged on the basilar membrane in an irregular manner along its length. This is not compatible with the observed delicate tuning of the ear Wien (115 116) has objected to the Helmholtz theory of hearing on similar theoretical reasons stating that the damping coefficient of the basilar membrane as demanded by Helmholtz s theory is not compatible with the observed response of the ear after a tone is switched off Therefore the basilar membrane has to be ruled out as a suitable resonator performing the observed frequency analysis.

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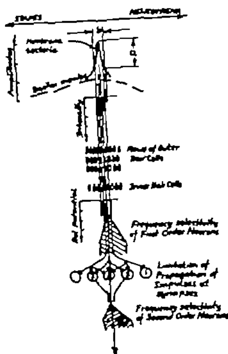


Fig 15 Schematic illustration of frequency limitation of transmitted band. Only the range b will send signals, where the amplitudes are above the minimum required. For sake of clarity phase shift between oscillations of tectoria and reticularis is not shown. The reticular lamina will only be involved at elevated frequencies. The band of signals generated will be narrowed-in at first and at second order neurons and at synaptic connections. The figures in the circles illustrate the number of stimuli arriving at the respective connection.

damping is less pronounced because there are no additional elements arranged on it, like those found on the basilar membrane. Contrary to the basilar membrane its design is very precise, showing very gradual variation of dimensions, as discussed earlier and of masses, with no irregularities or factors exercising an adverse influence. Therefore this theory does not attract the criticism raised by Wien (loc. cit.) against Helmholtz's theory of hearing.

It seems, therefore, possible to suggest an overall frequency analysing mechanism as shown in Fig. 15 Only that sector of the cochlear partition will transmit a signal where the relative displacement, combined with the generation and transmission efficiency of members involved, is above the minimum required. First as well as second order neurons have been found to be frequency selective (34 61 72–76), therefore, the transmitted signal band will be narrowed in. In addition to this a limitation of signal-band propagation will take place at synaptic connections (61–63) as the further transmission of the signal depends on the strength and on the number of signals arriving at synaptic connections.

D Area of maximum stimulus

According to this theory the area of maximum electrical activity (117) corresponds to the resonance peak of the envelope of deflections of the tectoria, which is in accordance with observations. This is not so in other theories where eddies (118–124) are assumed to be responsible for the auditory sensation, as they would appear on the apical side of the deflection.

The width of the area of maximum electrical activity corresponds to the resonance peak of the tectorial membrane, but not to the flat envelope of the amplitudes of the basilar membrane.

E. Sensitivity to frequency shift

The ear is sensitive to a frequency change of about 3 cps (125) This corresponds to a longitudinal shift along the cochlear partition of about $1.3 \times 10^{-}$ cm, as may be established from observations (117 124 126–130) on which the curve for the human ear in Fig. 13 is based. The envelope of the displacement of the basilar membrane is not in a position to explain this phenomenon, because the amount of shift is only about one five hundredth of the length of the extremely flat peak of the deflection exceeding 50% of the relative maximum amplitude. Furthermore, this shift in the longitudinal direction, which is detected by the ear is only about one-sixth of the height of the lattice girder (cf. section B). The observed frequency-shift sensitivity of the hearing instru-

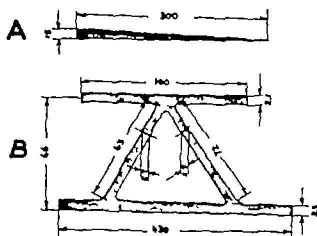


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In the light of this theory it is evident that differences in tuning will occur if the natural frequency diagrams of the two ears are not congruent, because there is, e.g. an inherited difference between the two sides, like an asymmetric face. If the oscillating structures, in particular the membrana tectoria, are not the mirror image of each other different signals will be fed by each ear into the higher neural pathways. Pathological deviations in construction between the two ears are to be expected.

H. Intensity sensitivity of ear as function of frequency

The very substantial difference in sensitivity at various frequencies at the absolute threshold of hearing (4 28 133) is obvious when considering the physical behaviour of the structures in the light of this approach to the theory of hearing. The lattice girder incorporating the basilar membrane acts as a counter bearing or partner of the tectoria as regards relative movements, and will show a deflection which increases approximately with the third power of the width of the membrane. The alteration of deflection resulting herefrom will accordingly affect the counterbearing capacity of the basilar membrane and therefore the relative movements, as will be discussed under section k.

Using the width of the basilar membrane as shown in Table II and extrapolating the curve towards the stapes, it is seen that the ratio of width for a place, near to the stapes, to a place near to the helicotrema is about 1 12. As the deflection depends about on the third power of the span, the ratio of deflections of 1 in 1 728 results.

Converting the deflection or amplitude ratio to a logarithmic scale as used for the decibel notation in hearing we get.

$$N_{(db)} = 20 \log a / a_2, \qquad (8)$$

where a and a_2 are the amplitudes now inserting the above ratio of deflections in formula (8), it is seen that a difference in sensitivity of about 65 db results, which is in consonance with actual observations.

This calculation does not allow for a number of minor factors, like arrangement of the organ of Corti which is not located always at the same distance from the center of the basilar membrane.

The dimensions given in Table II show further that the thickness of the basilar membrane and therefore its moment of inertia, as well as that of the other important parts of the architectural structure, change as one moves from the stapes to the helicotrema. Attention is also invited regarding the deformation of the tissues under the force of the knife of the microtome, discussed earlier and to a certain amount of loose play in the basilar membrane towards the apex factors which have to be taken into account as they affect the deflection of the basilar membrane. Certain deviations from the sensitivity difference calculated above are therefore, to be expected.

Further energy will be consumed during transmission, which, together with the observed loosed play will also influence the absolute threshold of hearing, considering that the very small volume of lymph displaced by the footplate of the stirrup at low sound intensities will be more effective near the stapes, where the deflections of the basilar membrane and of the tectorial membrane are smaller

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However the anisotropic mechanical properties of the tectorial membrane with very little coupling in the longitudinal direction (cf Fig 2) are in a position to explain this *property of the* ear because even if for demonstration purpose the tectorial membrane is not considered to be anisotropic its *moment* of inertia is about one fiftieth of that of the lattice girder Under uniformly distributed static loads, which are equal for both structures, the deflection of the tectorial membrane will be about the 10^3 fold of that of the girder depending on the place along the cochlear *partition, i e* on the span and *moment* of inertia at each place This value obtained from this investigation, compares *favourably* with the observations of Békésy (96) In spite of the fact that the last calculation can only be considered as a first approximation (considering the problem of exact evaluation of the deflection of the very intricate lattice-girder which follows a complex curve in space and is of *anisotropic organic material) it illustrates a* further observation in accord with the theory

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It was found in the course of the experiments that the width of the basilar membrane from the stapes to the helicotrema does not follow the same pattern for the various inner ears investigated but that there are some deviations. Such differences are probably inherited, *reminding of similarities between outer* ears, noses, faces, etc. of related persons and pronounced differences between such parts of non related persons.

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In the light of this theory it is evident that differences in tuning will occur if the natural frequency diagrams of the two ears are not congruent, because there is, e.g. an inherited difference between the two sides, like an asymmetric face. If the oscillating structures, in particular the membrana tectoria, are not the mirror image of each other different signals will be fed by each ear into the higher neural pathways. Pathological deviations in construction between the two ears are to be expected.

H. Intensity sensitivity of ear as function of frequency

The very substantial difference in sensitivity at various frequencies at the absolute *threshold* of hearing (4 28 133) is obvious when consider ing the physical behaviour of the structures in the light of this approach to the theory of hear ing. The lattice girder incorporating the basilar membrane acts as a counter bearing or partner of the tectoria as regards relative movements, and will show a deflection which increases approximately with the third power of the width of the membrane The alteration of deflection resulting herefrom will accordingly affect the counterbearing capacity of the basilar membrane and therefore the relative movements, as will be discussed under section K.

Using the width of the basilar membrane as shown in Table II and extrapolating the curve towards the stapes, it is seen that the ratio of width for a place, near to the stapes, to a place near to the helicotrema is about 1 12. As the deflection depends about on the third power of the span, the ratio of deflections of 1 in 1 728 results.

Converting the deflectron or amplitude ratio to a logarithmic scale as used for the decibel notation in hearing we get:

$$N_{(db)} = 20 \log a_1/a_n \quad (8)$$

where a and a are the amplitudes now inserting the above ratio of deflections in for mula (8) it is seen that a difference in sensitivity of about 65 db results, which is in consonance with actual observations.

This calculation does not allow for a number of minor factors, like arrangement of the organ of Corti which is not located always at the same distance from the center of the basilar membrane.

The dimensions given in Table II show further that the thickness of the basilar membrane and therefore its moment of inertia as well as that of the other important parts of the architectural structure change as one moves from the stapes to the helicotrema. Attention is also invited regarding the deformation of the tissues under the force of the knife of the microtome, discussed earlier and to a certain amount of loose play in the basilar membrane towards the apex, factors which have to be taken into account as they affect the deflection of the basilar membrane. Certain deviations from the sensitivity difference calculated above are, therefore, to be expected.

Further energy will be consumed during transmission, which, together with the observed loosed play will also influence the absolute threshold of hearing, considering that the very small volume of lymph displaced by the footplate of the stirrup at low sound intensities will be more effective near the stapes, where the deflections of the basilar membrane and of the tectorial membrane are smaller

than at the apical side of the cochlear partition.

I Influence of age on the upper frequency limit

Equation (6) for the natural frequency of the architectural structure shows, that a decrease of Young s modulus will lower the frequency of the system It has been shown for some tissues of the body (134) that Young s modulus decreases considerably with advancing age Therefore, the tectorial membrane will be affected and the upper frequency limit will be reduced

It is to be expected that ageing of other parts of the system in particular of those responsible for the generation and conduction of the electric signals will enhance the effect.

K. *Influence of age on the sensitivity of the ear*

The reduction of sensitivity of the ear with advancing age was, so far mostly attributed to reduced efficiency of transmission of energy e g. to otosclerosis and/or to a hitherto obscure deterioration of the neural part including transducers

In the light of this theory it is, however evident that even in the absence of otosclerosis or neural deterioration the sensitivity will be reduced As Young s modulus of the basilar membrane and of the lattice-girder reinforcing it will be affected by the process of ageing, its support to the architectural structures will be reduced and the amplitudes of the basilar membrane will increase Thereby the relative displacement of the membrana tectoria and of the cuticular plate at the top of the hair cells against their lower end is reduced. A similar decrease in Young s modulus applies to the membrana reticularis und to the phalangeal cells, which support the upper and lower side of the transducers. This means that the intensity of a tone, required to cause a sensation at the absolute threshold of hearing must increase more and more with advancing age

L. Effects of a localized lesion of the basilar membrane

It has been pointed out that a localized lesion of the basilar membrane should not change the hydrodynamic conditions or the envelope of the amplitudes of the basilar membrane, or eddies, or shearing forces—all of which have been advanced to explain the properties of the ear at some time—in such a way that there is no tonal sensation for the particular frequency range, if stimulation due to such causes would take place

However adopting the approach suggested here, it is seen that at such places of lesion the base of the structure fails and will follow the oscillations of the membrana tectoria, exercising little or no resistance which means that the relative displacement will be greatly diminished and very small or insufficient strain in the transducers will result

M *Proof that the amplitudes of the membrana tectoria are sufficient to cause a sensation*

When trying to explain the auditory sensations with the help of the envelope of deflections of the basilar membrane or of the cochlear partition as a whole, as done so far the absolute amount of deflection of such structures at the absolute threshold of hearing or even at low sound levels presents a major problem (25 135 139)

For a tone of e g. 200 cps at the absolute threshold of hearing, the amplitude of the basilar membrane is about 10^{-11} cm (25) and for a similar frequency at the threshold of feeling only about 10^{-4} cm (112)

Any theory will therefore, have to explain how the extremely small deflections at the absolute threshold of hearing are sufficient to cause a sensation

The results of studies of the relative stiffness of various membranes of the cochlear partition carried out by Békésy (96) and the results of this investigation prove that there is a pronounced difference in elasticity of up to

second exponential order under static loads, between the tectorial membrane and the lattice girder including the basilar membrane. Békésy's tests, using small magnifications in the microscope and heavy loads, furthermore show a decreasing elasticity towards the stapes, although he mentions only the large variation of elastic properties of the basilar membrane.

As the difference of elasticity between the tectorial and the basilar membrane is of second exponential order under static load, the magnification due to resonance of the tectorial membrane will change this difference to about third exponential order i.e. if the amplitude of the basilar membrane for a 200 cps tone at the absolute threshold is about 10^{-11} cm, equal to 10 μ the amplitude of the membrana tectoria and of the transmitter pins, and, therefore, of the diaphragm closing the top of the transducers, will be about 10 μ The relative displacement, i.e. the elongation or compression of the hair-cells will, therefore, be about 10^{-} μ as the amount to be deducted (10^{-7} μ) can be disregarded.

In this approach, the detailed behaviour of the structure, like the compensation due to compression of the phalangeal cells, sideways deflection of the transducers, particularly towards the apex where they are longer has been simplified for the sake of a first approximation, and as the design of the system suggests that such influences are of secondary importance.

As the compressibility of the cytoplasm in the transducers is extremely small (cf. bulk modulus for liquids), the shape of the transducers will change and their outer membrane as well as the small and large nerve endings will be subjected to strain.

Strain on the transducers due to an upward deflection of the tectorial membrane will be considered first. As the total relative deflection at given conditions is about 10^{-4} μ (assuming an effective length of transducer wall acting on a nerve ending of 10 μ) the maximum axial elongation which will approximately influence a single terminal area of one nerve fiber (effective portion of transducers length over which nerve endings are to be found) is about 4×10^{-8} μ

Considering compression of transducers now if the volume of one transducer which remains constant, is V and its length before and after compression is l_0 and l_1 (i.e. the length of the tubular transducer is l_0, and l_1 is the length reduced by the stroke of the piston) and the respective diameters of the transducers are d_0 and d_1, it is seen that

$$V = \frac{\pi}{4} d_0^2 \ l_0 = \frac{\pi}{4} d_1^2 \ l_1$$

which results in.

$$d_1 = d \sqrt{\frac{l}{l}}$$

Inserting $d_0 = 7.5$ micron, $l_0 = 25$ micron, and $l_1 = 25 - 10^{-4}$ micron, d_1 will be about 7.50024 μ or the increase in circumference of the transducers

$$\Delta U = \Delta d \ \pi = 0.0000752\,\mu.$$

Considering the circumferential elongation of an effective length of 10μ as above, the standard elongation will be about $3.2 \times 10^{-}$ μ. It is seen that the values for compression and elongation are rather similar as are the recorded corresponding amplitudes of the aural microphonics.

Now it will be interesting to see what stress may be approximately expected in the macromolecular substance of the transducer wall and of the nerve fiber

At the very small amplitudes discussed here it is assumed that Hooke's law may be applied to obtain an approximate result. Then the strain is

$$\varepsilon = \alpha \ \sigma \qquad (10)$$

in which α is the reciprocal value of Young's modulus *(E)*, and σ is the stress. From

$\alpha = 1/E$, and

$\varepsilon = \Delta l / l = (l - l_0)/l_0$

$$\sigma = (E \ \Delta l)/l_0 \qquad (11)$$

than at the apical side of the cochlear partition.

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membrana tectoria at the absolute threshold are only amounting about $10^{-}\ \mu$ as compared to a span of the membrane of about 300μ which gives a ratio of deflection to length of the vibrating member of about 1 in 3×10^{6} or for a 60 db tone only about 1 in $3\ 10^{3}$ Therefore, in the subsequent treatment of the problem only a linear system will be considered.

The complete integral of the above equation is

$$y = Ae^{-\,\,t}\sin(\beta t+\varrho_0)\frac{a}{\sqrt{(q-\omega^2)^2+p^2\omega^2}}\sin(\omega t+\varrho\ )\qquad(13)$$

in which ϱ is to be obtained from the particular solution of the differential equation of forced oscillations, viz.,

$$\tan\varrho = \frac{M}{N},\ \text{in which}\ M = \frac{-p\,a\,\omega}{(q-\omega^2)^2+p^2\omega^2}$$

$$N = \frac{a(q-\omega^2)}{(p-\omega^2)^2+p^2\omega^2}$$

the initial phase being ϱ_0.

The first term of the sum on the right side of (13) (the solution of the homogeneous equation) represents damped oscillations and will diminish with increasing time *(t)* and, therefore, after an interval of time the second term representing forced oscillations will acquire prime importance (cf. 109 150, 151)

Such a combination of transient and forced vibrations may however be expected to produce beats, the frequency of which is the difference between $/2$ and $\beta\ /2\pi$ where β is the frequency of free oscillations of the system, which will be damped much faster the more pronounced the damping of the system is.

Such beats caused by the transient and forced vibration are actually to be observed (109) and speak, therefore, very strongly in favour of the existence of a peripheral oscillating system, and support this theory

It will now be of interest to investigate the amplitude of forced oscillations as function of ω for different values of p Denoting the amplitude of forced vibrations by $D(\omega)$ it will be seen that

$$D(\omega) = \frac{a}{\sqrt{(q-\omega^2)^2+p^2\omega^2}}\qquad(14)$$

For $p = 0$ (no resistance) the frequency of free oscillations of the system will be equal to the natural frequency If $q = \beta_1^2$ the equation will become

$$D(\omega) = \frac{a}{\sqrt{(\beta_1^2-\omega^2)^2+p^2\omega^2}}\qquad(15)$$

or introducing the ratio $\omega/\beta_1 = \lambda$ i.e. the ratio of the frequency of the force to the frequency of free oscillations of the system in order to be able to develop the resonance curve for forced, damped oscillations, the new equation will be

$$D(\omega) = \frac{a}{\beta^2\sqrt{\left(1-\frac{\omega^2}{\beta_1^2}\right)+\frac{p^2\omega^2}{\beta_1^2\beta_1^2}}}\qquad(16)$$

or

$$D(\lambda) = \frac{a}{\beta_1^2\sqrt{(1-\lambda^2)^2+\gamma^2\lambda}}\qquad(17)$$

if γ is the ratio of p to β

The maximum of this function will be reached for that value of λ for which the square of the denominator has a minimum, and the minimum of $\sqrt{(1-\lambda^2)^2+\gamma^2\lambda}$ is reached when

$$\lambda = \sqrt{1-\gamma^2/2}\qquad(18)$$

and when this is equivalent to

$$\gamma\sqrt{1\ \ \gamma^2/4}\qquad(19)$$

Therefore, the maximum amplitude will be

$$D_{max} = \frac{a}{\beta^2\sqrt{1-\gamma^2/4}}\qquad(20)$$

It is now possible to construct the above mentioned graph of the function $D(\lambda)$ in a general way taking a and β_1 as unity for the sake of definiteness.

The curves obtained in this way are reso-

is obtained, from which the stress for unit cross section may be calculated

Values for Young s modulus for transducer wall and nerve fibers thereon are not known and therefore they may be estimated with the help of data for organic materials (140–142) Values for (E) given are 10^5 kg/cm^2 for most woods for forces parallel to the grain, and 2 to 3×10^4 kg/cm^2 for polystyrol and polyvinyl chloride Human sinews possess a tensile strength of 600 kg/cm^2 to 1 200 kg/cm^2 (31 143) comparable to the average value for pine of about 900 kg/cm^2 elm (800 kg/cm^2) or poplar (770 kg/cm^2) (140–142) As however the tissues involved are very delicate, the calculations will not only be based on above values, but on a third alternative with only one tenth of the value for polystyrol

Using the standard axial elongation determined above ($4 \times 10^{-5}\mu = 4 \times 10^{-9}$ cm) for Δl and for l the standard length of $10\mu = 10^{-3}$ cm the following stress (σ in kg/cm^2) for the three values of E stated above is obtained

(a) *wood and sinews*

$\sigma = 10^5 \times 4 \times 10^{-9} \times 10^3 = 0\ 4$ kg/cm^2

(b) *polystyrol*

$\sigma = 2 \times 10^4 \times 4 \times 10^{-9} \times 10^3 = 0\ 08$ kg/cm^2

(c) *One tenth of polystyrol i e one-fiftieth of sinews*

$\sigma = 2 \times 10^3 \times 4 \times 10^{-9} \times 10^3 = 0\ 008$ kg/cm^2

It is evident that even the smallest amount of stress of 8 g/cm^2 will produce a sensation, because a much smaller load applied slowly to skin area of medium sensitivity still produces a sensation. In the case of the cochlear partition *the change of load is very rapid, therefore,* the minimum stress required to produce a sensation will be lower according to observations on human skin sensitivity (cutaneous touch) (144–147) In addition the innervation of transducers is more extensive than that of the skin, and also their environmental conditions *are more favourable for ion migration*

Therefore even when considering factors which lower the efficiency it appears that the amplitudes at the *absolute threshold of hearing* are adequate to cause an auditory sensation, on the basis of the physical performance advanced in this theory

N Masking, critical band of masking and of beats

Before discussing phenomena observed in connection with masking it may be stated that *masking is of peripheral origin,* which is confirmed by the observation that masking does not occur when the masking tone is applied to one ear and the masked to the other one (148 149) The little amount of masking observed under such conditions can be ascribed to a great extent to the transmittance of vibrations around the head (112)

It has been observed that low frequency tones mask high frequency tones effectively whereas the *efficiency is much less* in the opposite case This will now be investigated in the light of this theory which suggests a resonator system and will therefore have to follow the law of mechanical vibrations.

In this system the movement is predominant in one direction (relative displacement of the *tectorial membrane*) *therefore, it may be* treated as having one degree of freedom only

The vibrations of the membrana tectoria are subject to elastic forces within this structure (spring constant) and the resisting forces causing damping, which are the fluid resistance (viscosity) and the internal friction. The equation of forced, damped oscillations has the form

$$\ddot{y} + p\dot{y} + qy = a \sin \omega t \qquad (12)$$

Cases where the vibrating system is linear over a wide range of amplitudes are very rare even if the material exposed to vibrations is not organic. Observations (96) *indicate* however that within most of the range of physiological sound intensities the characteristics of the ear are *nearly linear* which seems reasonable in the light of this theory as the deflections of the

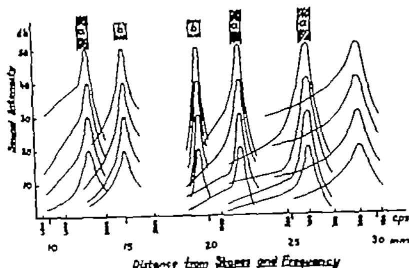


Fig 17 Redrawn masking data, using linear scale for the distance from the stapes as abscissa and showing the frequency reception places along it. The ordinate shows the sound intensity

If available data is re-evaluated in this way resonance curves result. It appears from their shape that damping is small in the central region, which is in agreement with the observation that frequency discrimination of the ear is best there.

Critical bands of masking (*a*) and range of beats (*b*) are shown. They show good agreement with the resonance peaks.

step. Therefore, present theories fail to explain this physical property of the ear

However such sensations, sometimes called quantification seem reasonable at the absolute threshold of hearing, if considered in the light of this theory The transducers are arranged in distinct rows, and the tectorial membrane possesses segmented anisotropic properties. Therefore, a gradual change in intensity or frequency will at a certain level reach the minimum amount of deflection required to switch a further group of transducers in a further row or a different segment on or off, and thereby produce a stepwise change of sensation.

The change in distance between rows, as function of distance from stapes as well as number of transducers energized explains the somewhat bigger steps at the low frequency side of the cochlea. Due to the physical properties of the tectorial membrane, the resonance peak of it becomes wider towards the low frequency range. This explains why the ear is not as sensitive in this range to frequency shifts as compared to the region closer to the stapes.

P Acoustic trauma

Sounds of excessive intensity lead to damage of certain transducers. So far the problem has been mostly treated in a general way but properly stated, three physical phenomena are concerned, for none of which a satisfactory explanation is available if the present theories of hearing are applied. Hereunder each phenomenon will be discussed separately

(1) *Width of Damaged Band of Transducers*

It is obvious that a tone of excessive intensity affects only a narrow segment of the cochlear partition (109). Neither the flat peak of the envelope of displacement of the basilar membrane nor the area affected by eddies are in a position to furnish an explanation.

Considering, however the functioning of the anisotropic tectorial membrane and the resulting resonance curve when stimulated by a tone of excessive intensity on the basis of this theory its pronounced resonance peak explains why only a narrow segment of transducers is affected (cf Fig. 17)

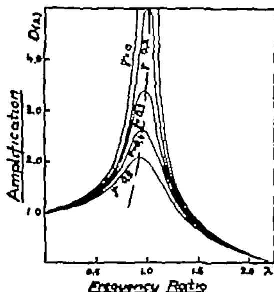


Fig 16 Resonance curves, which show on the ordinate the amplification of vibration due to resonance for various amounts of damping (γ), and on the abscissa the ratio of the frequency of the force to the frequency of the system (λ). The maximum shifts to the left if the damping increases. The amplification is more pronounced for $\lambda < 1$ compared to $\lambda > 1$

nance curves (cf Fig. 16) and are required to determine whether the system under study behaves as demanded in connection with forced, damped oscillations.

It is seen (Fig. 16) that the amplification effect is more pronounced for $\lambda < 1$ which means, returning to the problem of the cochlear partition, on the high frequency side of the force i e on the side of the stapes This, therefore, confirms the observation, according to which masking by a tone of lower frequency is more effective and also that the envelope decreases slower towards the stapes than in the direction of the helicotrema

If a system behaves like a series of resonators as confirmed by above discussions, it seems reasonable that harmonics should occur if the intensity of the tone is sufficiently raised. This is the case (149 152) and furnishes a further confirmation of this theory

It is observed further that the critical band of masking, as well as the band where beats occur are far narrower than the flat peak of deflections of the basilar membrane, and the latter therefore falls also for this reason to furnish an explanation for these observations.

If however the method used mostly when drawing masking diagrams is abandoned, which shows the influence of masking by indicating the sensation level of the primary tone on the abscissa, the threshold shift on the ordinate and the frequency of the secondary tones as parameters, using separate diagrams for each frequency (131 149 152), and the method shown in Fig. 17 is adopted, good agreement between the resonance peak of the tectorial membrane and the critical band of masking is found This means that two rather old observations on masking (149) and on critical bands (127 128 153), confirm this theory and the shape of the resonance curves.

Helmholtz (154) suggested that we hear beats due to imperfect frequency analysing properties of the cochlear mechanism because the two frequency components of the stimulus affect overlapping segments of the basilar membrane.

As the peak of the envelope of the amplitudes of the basilar membrane is very flat, beats should cover a much wider frequency range than actually observed. On the other hand if one assumes that beats are caused by overlapping segments of the resonating tectorial membrane, as suggested by this theory the range where beats may occur becomes much narrower and agrees with observations, as demonstrated in Fig. 17

O Stepwise auditory sensations (quantification)

This phenomenon (4 28 131 155 156) cannot be explained if only the envelope of amplitudes of the basilar membrane or eddies are considered, as both change gradually and not in step-wise fashion. Further any longitudinal shift along the extremely flat peak of the envelope of oscillations of the basilar membrane of the observed amount (cf C and E) does not practically involve a change of amplitude of adjoining areas of the basilar membrane and, therefore, fails to explain a sudden sensational

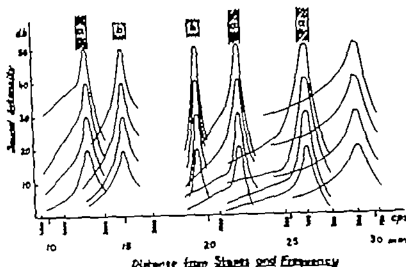


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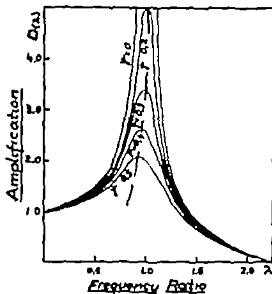


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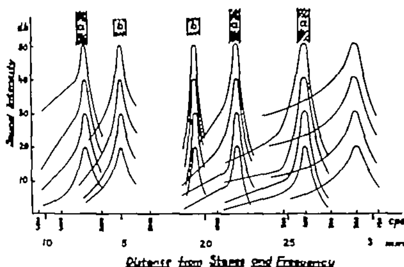


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step. Therefore, present theories fail to explain this physical property of the ear.

However such sensations, sometimes called quantification seem reasonable at the absolute threshold of hearing, if considered in the light of this theory. The transducers are arranged in distinct rows, and the tectorial membrane possesses segmented anisotropic properties. Therefore, a gradual change in intensity or frequency will at a certain level reach the minimum amount of deflection required to switch a further group of transducers in a further row or a different segment on or off, and thereby produce a stepwise change of sensation.

The change in distance between rows, as function of distance from stapes as well as number of transducers energized explains the somewhat bigger steps at the low frequency side of the cochlea. Due to the physical properties of the tectorial membrane, the resonance peak of it becomes wider towards the low frequency range. This explains why the ear is not as sensitive in this range to frequency shifts as compared to the region closer to the stapes.

P. Acoustic trauma

Sounds of excessive intensity lead to damage of certain transducers. So far the problem has been mostly treated in a general way but properly stated, three physical phenomena are concerned, for none of which a satisfactory explanation is available if the present theories of hearing are applied. Hereunder each phenomenon will be discussed separately.

(1) *Width of Damaged Band of Transducers*

It is obvious that a tone of excessive intensity affects only a narrow segment of the cochlear partition (109). Neither the flat peak of the envelope of displacement of the basilar membrane nor the area affected by eddies are in a position to furnish an explanation.

Considering, however the functioning of the anisotropic tectorial membrane and the resulting resonance curve when stimulated by a tone of excessive intensity on the basis of this theory its pronounced resonance peak explains why only a narrow segment of transducers is affected (cf. Fig. 17).

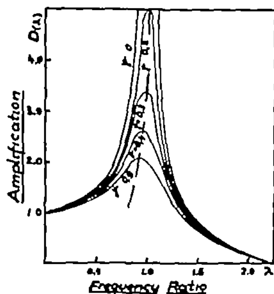


Fig 16 Resonance curves, which show on the ordinate the amplification of vibration due to resonance for various amounts of damping (γ), and on the abscissa the ratio of the frequency of the force to the frequency of the system (λ). The maximum shifts to the left if the damping increases. The amplification is more pronounced for $\lambda < 1$ compared to $\lambda > 1$

nance curves (cf Fig. 16) and are required to determine whether the system under study behaves as demanded in connection with forced damped oscillations.

It is seen (Fig. 16) that the amplification effect is more pronounced for $\lambda < 1$ which means returning to the problem of the cochlear partition, on the high frequency side of the force i.e. on the side of the stapes This therefore confirms the observation according to which masking by a tone of lower frequency is more effective and also that the envelope decreases slower towards the stapes than in the direction of the helicotrema

If a system behaves like a series of resonators, as confirmed by above discussions, it seems reasonable that harmonics should occur if the intensity of the tone is sufficiently raised This is the case (149 152) and furnishes a further confirmation of this theory

It is observed further that the critical band of masking, as well as the band where beats occur are far narrower than the flat peak of deflections of the basilar membrane and *the latter therefore falls also for this reason to furnish an explanation for these observations*

If however the method used mostly when drawing masking diagrams is abandoned, *which shows the influence of masking* by indicating the sensation level of the primary tone on the abscissa the threshold shift on the ordinate and the frequency of the secondary tones as parameters, using separate diagrams for each frequency (131 149 152), *and the* method shown in Fig. 17 is adopted, good agreement between the resonance peak of the *tectorial membrane and the critical band of* masking is found This means that two rather old observations on masking (149) and on critical bands (127 128 153) confirm this theory and the shape of the resonance curves.

Helmholtz (154) suggested that we hear beats due to imperfect frequency analysing properties of the cochlear mechanism because *the two frequency components of the stimulus* affect overlapping segments of the basilar membrane

As the peak of the envelope of the amplitudes of the basilar membrane is very flat, beats should cover a much wider frequency range than actually observed On the other hand if one assumes that beats are caused by overlapping segments of the resonating tectorial membrane, as suggested by this theory the range where beats may occur becomes much narrower and agrees with observations, as demonstrated in Fig. 17

O Stepwise auditory sensations (quantification)

This phenomenon (4 28 131 155 156) can not be explained if only the envelope of amplitudes of the basilar membrane or eddies are considered, as both change gradually and not in step-wise fashion Further any longitudinal shift along the extremely flat peak of the envelope of oscillations of the basilar membrane of the observed amount (cf C and E) does not practically involve a change of amplitude of adjoining areas of the basilar membrane and therefore fails to explain a sudden sensational

The amplitude of forced, damped oscillations is

$$A = \frac{Q}{k\sqrt{\left(1-\frac{\omega^2}{\beta^2}\right) + \frac{c^2\omega^2}{k^2}}} \quad (24)$$

Here Q is the maximum distorting force, k is the spring constant, and c is the coefficient of damping. Rewriting we get:

$$\left(1-\frac{\omega^2}{\beta^2}\right) = \left(\frac{Q}{k\ A}\right) - \left(\frac{c\omega}{k}\right) \quad (25)$$

or

$$\beta_1 = \frac{\omega}{\sqrt{1-\sqrt{\frac{Q^2 - A^2c^2}{k\ A^2}}}} \quad (26)$$

As the efficiency of the transducer system can be regarded to be constant along the cochlear partition, the relative amplitude of the tectorial membrane acting on them remains constant (cf. chapter H)

However the hydrodynamic form of sound energy suffers loss during transmission along the cochlear partition. This is due to the dissipative viscous forces of the liquid, which increases with the length of the path, i.e. towards the places of reception of the low frequencies. Furthermore, the amplitudes of oscillations of the liquid, required to produce a certain effect, also grows towards the apex due to the increased deflection of the basilar membrane there. These viscous forces, therefore, also grow and dissipate more hydraulic energy. Moreover energy is consumed by the cochlear partition due to the internal friction of all parts displaced.

Therefore A remains constant, while Q decreases as given by the formula (26) above: the value of the denominator increases and the angular frequency β will be lowered. This is equivalent to shifting the place of maximum response of the system towards the place of reception of lower frequencies, in agreement with observations.

A similar reduction of the natural frequency of a resonator has been cited by A. Wood (109)

Of course, the actual characteristics will incorporate both effects, and on the basis of the theory advanced here some further small shifts may be expected. For instance, in case the damping of the oscillating system does not change in an identical way for both ears as a function of sound intensity not only shifts in tuning will differ correspondingly for both ears, but also the place of acoustic trauma will be affected.

Q. Stimulation of transducers by flow of lymph

Neubert (43) has suggested, that the hairs of the hair-cells are acted upon by the lymph, which is forced out between the tectorial membrane and the upper side of the organ of Corti, due to differential displacement of the membrane of Reissner against the basilar membrane.

This investigation disproves this theory because there is a connection between the tectorial membrane and the transmitter pins contrary to his assumptions. Furthermore, the tectorial membrane is at its outer edge firmly attached to the organ of Corti. There is, therefore, no slit-nozzle through which liquid may be forced.

As regards the suggestions concerning the generation of sensation due to eddies, many reasons have been given which contradict this possibility. It is suggested to regard eddies, if occurring at all at low or normal sound intensities as side effects they are rather undesirable because they consume valuable energy.

R. Comparison to vestibular apparatus

The cupola of the vestibular apparatus acts like a swinging door which is hinged on the transducers, and is moved when the head turns, due to the inertia of the lymph in a semicircular canal, which has a tendency to remain stationary. These canals are similar from fish to man and direct observations have been

(2) *Transverse Localization of Damage to Transducers*

It has been found that the external transducers are more susceptible to injury due to excessive intensity of a tone, than the inner ones (56 157 158) It is obvious that the outer part of the tectorial membrane is deflected more than the inner side Therefore an excessive strain on the transducers will happen there first

(3) *Longitudinal Localization of Damage to Transducers*

Experiments which expose the ear to high sound intensity resulting in the fatigue of the ear (159 160) or to excessive intensities causing damage (*109 161–165*) produce a phenomenon for which no interpretation has been offered so far

It is found that the place along the cochlear partition which has been affected by the frequency of excessive intensity does not correspond to the place of reception at normal intensities. An upward shift has been observed for about $f > 800$ cps, a downward shift for about $f < 800$ cps.

First the phenomenon of an upward shift will be studied applying the physical basis of this theory

As stated earlier (loc. cit.) the ear mechanism shows linear properties for the normal intensity range Therefore considerations will be based on such behaviour

Reverting to equation (18)

$$\lambda = \sqrt{1 - \gamma^2/2},$$

in which $\lambda = \omega/\beta_1$ and $\gamma = p/\beta_1$ Inserting these expressions we get

$$\omega/\beta_1 = \sqrt{1 - p^2/2\beta_1^2} \tag{21}$$

and solving this equation for β_1 the angular frequency of the oscillator will be

$$\beta_1 = \sqrt{\omega^2 + p^2/2} \tag{22}$$

where p is the resistance

This resistance is normally assumed to be proportional to the velocity at such small amplitudes (110 141) where the velocity depends on the amplitude if the frequency of the o lations is kept constant.

Now it is evident that the amplitude of tectorial membrane and of other oscilla parts of the system will increase when sound intensity is raised. This will be accon nied by an increase in resistance, which res in a shift of the place of maximum respo of the oscillating system In this connect attention is invited to Fig. 16 and it is s that the maxima of the resonance curves s towards the left, in the direction of decreas *λ if γ increases.*

In Table V the natural frequency of ma mum response of the system has been eva ated for a constant frequency of the force (of 1 000 cps The damping (γ) has be varied between 0 and 0 6 As discussed earl $n_f = \omega/2\pi$ and $n_e = \beta_1/2\pi$ Inserting in equ tion (22) one obtains

$$n_e = \sqrt{(2\pi n_f)^2 + p^2}/2\pi, \tag{2$$

in which p is equivalent to $2\pi\beta_1\gamma$ and we g the values as given in Table V

Table V

n_f (cps)	1 000	1 000	1 000	1 000	1 000	1 0
γ[a]	0	0 2	0.3	0.4	0.5	0
p[b]	0	1 250	1 920	2 610	3 310	4 1
n (cps)	1 000	1 01	1 023	1 043	1 067	1 1
Δ cps ( )	0	1.2	2.3	4 3	6.7	

[a] Damping factor [b] Resistance units.

The resistance is however not necessaril proportional to the velocity In hydrodynamic the resistance is assumed to vary with th square of the velocity (110 et al) This wi increase the shift even more Due to the intri cate design of the system one must expect a rather complex behaviour of the characteristic of the system as the sound intensity is raised However the general characteristics will remain the same as discussed above and the theory is, therefore, in a position to explain the observation.

Now the theory will be tested as regards shift of place of damage towards the apex caused by an excessive low frequency sound intensity

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in which p is equivalent to $2\pi\beta_1\gamma$ and we get the values as given in Table V

Table V

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γ^a	0	0	0.3	0.4	0.5	0 6
p^b	0	1 [illegible]	1 970	610	3 010	4 100
n (cps)	1 000	1 012	1 023	1 043	1 067	1 100
Δ cps ( )	0	[illegible]	2.3	4 3	6.7	10

Damping factor [b] Resistance units.

The resistance is however not necessarily proportional to the velocity In hydrodynamics the resistance is assumed to vary with the square of the velocity (110 et al) This will increase the shift even more. Due to the intricate design of the system one must expect a rather complex behaviour of the characteristics of the system as the sound intensity is raised However the general characteristics will remain the same as discussed above and the theory is, therefore in a position to explain the observation

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Conclusions

The above discussion shows that this theory is capable of elucidating the physical properties of the ear better than any available theory It is seen that this is possible without any far fetched assumptions, arguments or obscure mechanism. On the contrary the physical behaviour of the cochlear partition as supported by this theory seems to be quite obvious, and each one of the many parts of the system can be assigned a plausible function.

It is seen that the most pronounced short coming of earlier theories is to consider the cochlear partition as one part, in spite of the fact that it contains a great number of precisely built elements. A further shortcoming is not to have obtained sufficient dimensional data to permit complete reproduction of the complex instrument within the cochlear partition. Finally attempts of an understanding of the instrument were faced with considerable difficulties because the instrument was not available intact but considerably distorted.

The approach adopted here, which led to the new theory suggests a considerable scope for further investigations, which can certainly lead to a more detailed understanding of the func tioning of this most complicated instrument of the human body

made on the ampullae in fish The function of the canals has been ascertained by experiments, with models, clinically and theoretically (145 166–178)

It has been established that the cochlea is the phylogenetically younger part of the labyrinthine system and has developed from the vestibular apparatus. There is no reason why nature should have changed the use of the hair cells, which is compression and elongation in the vestibular apparatus when the cochlea was developed It may be of interest that the same design of vestibular apparatus has been used by nature for about 400 million years, and that of the cochlea for only about one quarter of this time This shows that there was apparently little room for further improvement of the mode of action on the transducers, and it is difficult to think that this time tested design should have changed in mammalia. This, therefore also supports the theory advanced here

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Quantitativ-cytochemische Untersuchungen am Innenohr junger und seniler Meerschweinchen

VON

H. KRAUS

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VON

H KRAUS

Aus der Hals-Nasen-Ohrenklinik der Westfälischen
Wilhelms-Universität Münster/Westf. (D 44)
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1 Einleitung

Die Kenntnis der normalen und pathologischen Biochemie der häutigen Strukturen des Innenohres ist aus methodischen Gründen weitgehend auf morphologische (Übersichten: Beck, 1965 Spoendlin, 1966 a Iurato, 1967) und fermenthistochemische Beobachtungen (Vosteen, 1964 et al.) beschränkt. Für mikrochemische Analysen (Naftalin u. Mitarb., 1964 Iurato 1960 1962, Kuhn u. Mitarb 1968) und Stoffwechseluntersuchungen *in vitro* (Chou, 1963 Rauch, 1964 Yanagava, 1964 Mizukoshi u. Mitarb Matschinsky u. Mitarb., 1967) können Zellen und Rezeptoren nicht exakt isoliert werden. Aussichtsreich für die Fragestellungen der Innenohrbiochemie erscheinen daher Verfahren, die an Zellen neben der topischen Zuordnung Messungen im Mikrobereich gestatten.

In der vorliegenden Mitteilung wird über quantitativ-histochemische Untersuchungen am häutigen Innenohr von neugeborenen, reifen und senilen Meerschweinchen berichtet (Tab I). Mit Hilfe von Farbreaktionen wurde photometrisch der relative Eiweiß- und RNA-Gehalt im Cytoplasma fast aller Zellen des Innenohres bestimmt und mit historadiographisch ermittelten Werten des Eiweißumsatzes (Meyer zum Gottesberge, 1961 1965 Plester u. Mitarb., 1962, Koburg, 1961 Koburg u. Mitarb., 1962) verglichen. Die fehlende Parallelität zwischen beiden Größen weist auf eine hohe Intensität der ribosomalen Eiweißsynthese im Corti'schen Organ. Interferenzmikroskopisch wurde am membranösen Labyrinth die Substanzkonzentration als Trockenmasse pro Volumeneinheit und ultraviolett-mikrospektrographisch der Gehalt an aromatischen Aminosäuren im Vergleich zum färberischen Verhalten gegenüber einem anionischen Farbstoff und Gallocyanin-Chromalaun untersucht. Die Meßergebnisse erlauben Rückschlüsse auf die Physiko-Chemie des häutigen Innenohres und dessen Altern.

Verwendete Abkürzungen

RNA, Ribonukleinsäure; m-RNA, Boten-Ribonukleinsäure; t-RNA, transfer Ribonukleinsäure; r-RNA, ribosomale Ribonukleinsäure; GC, Gallocyanin-Chromalaun; NGS, Naphtholgelb S, UV Ultra-Violett; BM, Basilar-
[illegible]



Die Kenntnis der normalen und pathologischen Biochemie der häutigen Strukturen des Innenohres ist aus methodischen Gründen weitgehend auf morphologische (Übersichten: Beck, 1965 Spoendlin, 1966 a Iurato, 1967) und fermenthistochemische Beobachtungen (Vosteen, 1964 et al.) beschränkt. Für mikrochemische Analysen (Naftalin u. Mitarb 1964 Iurato, 1960 1962 Kuhn u. Mitarb., 1968) und Stoffwechseluntersuchungen *in vitro* (Chou, 1963 Rauch, 1964 Yanagava, 1964 Mizukoshi u. Mitarb Matschinsky u. Mitarb 1967) können Zellen und Rezeptoren nicht exakt isoliert werden. Aussichtsreich für die Fragestellungen der Innenohrbiochemie erscheinen daher Verfahren, die an Zellen neben der topischen Zuordnung Messungen im Mikrobereich gestatten.

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Verwendete Abkürzungen

RNA, Ribonukleinsäure; m-RNA, Boten-Ribonukleinsäure; t-RNA, transfer-Ribonukleinsäure; r-RNA, ribosomale Ribonukleinsäure; GC, Gallocyanin-Chromalaun; NGS, Naphtolgelb S, UV Ultra-Violett; BM, Basilarmembran.

1 Einleitung

Die Kenntnis der normalen und pathologischen Biochemie der häutigen Strukturen des Innenohres ist aus methodischen Gründen weitgehend auf morphologische (Übersichten: Beck, 1965 Spoendlin, 1966 *a* Iurato, 1967) und fermenthistochemische Beobachtungen (Vosteen, 1964 et al.) beschränkt. Für mikrochemische Analysen (Naftalin u. Mitarb., 1964 Iurato, 1960 1962 Kuhn u. Mitarb., 1968) und Stoffwechseluntersuchungen *in vitro* (Chou, 1963 Rauch, 1964 Yanagawa, 1964 Mizukoshi u. Mitarb Matschinsky u. Mitarb 1967) können Zellen und Rezeptoren nicht exakt isoliert werden. Aussichtsreich für die Fragestellungen der Innenohrbiochemie erscheinen daher Verfahren, die an Zellen neben der topischen Zuordnung Messungen im Mikrobereich gestatten.

In der vorliegenden Mitteilung wird über quantitativ-histochemische Untersuchungen am häutigen Innenohr von neugeborenen, reifen und senilen Meerschweinchen berichtet (Tab I). Mit Hilfe von Farbreaktionen wurde photometrisch der relative Eiweiß- und RNA-Gehalt im Cytoplasma fast aller Zellen des Innenohr bestimmt und mit historadiographisch ermittelten Werten des Eiweißumsatzes (Meyer zum Gottesberge, 1961 1965 Plester u. Mitarb., 1962, Koburg, 1961 Koburg u. Mitarb., 1962) verglichen. Die fehlende Parallelität zwischen beiden Größen weist auf eine hohe Intensität der ribosomalen Eiweißsynthese im Corti'schen Organ. Interferenzmikroskopisch wurde am membranösen Labyrinth die Substanzkonzentration als Trockenmasse pro Volumeneinheit und ultraviolett-mikrospektrographisch der Gehalt an aromatischen Aminosäuren im Vergleich zum färberischen Verhalten gegenüber einem anionischen Farbstoff und Gallocyanin-Chromalaun untersucht. Die Meßergebnisse erlauben Rückschlüsse auf die Physiko-Chemie des häutigen Innenohres und dessen Altern.

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2 Material und Methoden

VERSUCHSTIERE, HISTOLOGIE

Verwendet wurden zehn Albino-Meerschweinchen überwiegend weiblichen Geschlechtes aus einem händlereigenen Inzuchtstamm (Ernährung: Mohrrüben, Wasser ad lib.) von 200 bis 300 g Körpergewicht, d. h. an der Grenze zur Geschlechtsreife (Rogers, 1951 Altman 1964). Für interferometrische und uv mikroskopische Untersuchungen am membranösen *Labyrinth (Tab I) wurden zusätzlich fünf neu* geborene (50 bis 80 g, max Lebensalter 4 Tage) und drei senile Tiere (870 g, 670 g und 750 g) benutzt. Das Alter dieser Tiere lag über 5 Jahren als Zeichen der Senilität fanden sich ein *arcus senilis corneae* und typische Krallenveränderungen (Rogers, 1951 Reid u. Mitarb 1953). Innerhalb von 4 Min (Dekapitierung zu beliebigen Tageszeiten im Ätherrausch) wurde ein Drittel der knöchernen Zirkumferenz der Schnecke abgetragen, die einzelnen Schneckenwindungen *zur Fixierung* mittels einer Mikropipette mit 4% Formaldehyd 5% Trichloressigsäure (aa pH 0 6) bei Zimmertemperatur durchspült und das zurechtgeschnittene Felsenbein bei täglichem Wechsel in gleicher Lösung entkalkt. Für cytophotometrische Messungen der Eiweiß- und RNA Konzentration in Zellen von reifen Tieren wurde so kurz wie möglich entkalkt (4–6 Tage) für Untersuchungen am membranösen Labyrinth einheitlich für alle Altersklassen 10 bis 12 Tage lang. Nach Wässern in A dest (pH 5 4–5 8 zweimal 24 Std) wurden die Felsenbeine standardisiert über die aufsteigende Alkoholreihe in Paraffin Paraplast gerichtet eingebettet. Zentrale Schnitte parallel dem Modiolus von rd 7–8 μ Dicke wurden so aufgeklebt, daß ein Kontakt der Schnecke *selbst mit* Eiweiß-Glyzerin vermieden wurde. Für uv mikrospektrographische *Messungen* wurden von zwei Tieren je eine Schnecke und Achillessehne bei gleicher Fixierung bzw Entkalkung nebeneinander eingebettet, um eine möglichst gleichmäßige Schnittdicke zu erreichen.

Die Schnitte wurden *standardisiert in* Xylol entparaffiniert in absolutem Äthanol gespült, kurz luftgetrocknet und für die Interferometrie in Öl vom Brechungsindex $n_{D 20°} = 1\ 40$ (Fa. Cargille New York) und 1 45 (geschliffene Objektträger Deckgläschen von 0 18 mm ± 10% Dicke) für uv mikrospektrographische Messungen in wasserfreiem Glyzerin $n_{D 20°} =$ 1 45 *Deckgläser und Objektträger aus Quarz*) nach Färbungen in Öl von angeglichenem Brechungsindex (*$n_{20°} =$ 1 52–1 53*) eingedeckt.

FÄRBUNGEN

Zum photometrischen Nachweis chemischer Gewebsbestandteile wurden folgende Farbreaktionen verwendet (Tab I)

a) *Gallocyanin-Chromalaun*-Färbung (GC) bei pH 1 64 nach Sandritter u Mitarb (1963 *1966). Zur Kontrolle wurden Schnitte bei* 50 C 2 Std mit 0,5% RNAse (Worthington) in A dest mit *0 2 n NaOH auf pH 7 0 eingestellt* inkubiert und nach kurzem Spülen in A dest. gleichartig gefärbt

Bei Zellen gilt die Reaktion als spezifisch für die Phosphatgruppen der *DNS* die stöchiometrisch erfaßt wird (Sandritter u Mitarb 1966) *und der cytoplasmatischen RNA* (Mundkur 1961 Pakkenberg, 1962 Kiefer u Mitarb., 1966)

Tab. 1. *Übersicht der Messungen am membranösen Labyrinth und Zellen*

	Dichte (g/cm³) (Interferometrie)	Tyrosin-Tryptophan-Konzentration (UV-Mikrospektrographie)	Konzentration von Gesamteiweiß, RNA und Sulfatgruppen (Cytophotometrie nach Färbung mit NGS, GC und nach Millon)
Neugeborene	Basilarmembran	Basilarmembran Tectorialmembran Pfeilerkopf	0
Reife	Basilarmembran Tectorialmembran *Lig. spirale* *Pfeilerkopf*	Basilarmembran Tectorialmembran Pfeilerkopf	1) Basilarmembran Tectorialmembran *Lig. spirale* Pfeilerkopf 2) Cytoplasma von Zellen, vgl. Abb. 1
Senile Versuchstiere	Basilarmembran	*0*	0

Cytophotometrische Meßwerte der Farbstoffkonzentration sind ein relatives Maß für die RNA-Konzentration im Cytoplasma. Im extrazellulären Gewebe reagieren auch *Sulfatgruppen* der Grundsubstanz (Kiefer u. Mitarb. 1966).

b) *Naphtholgelb* S-Färbung (NGS) bei pH 2,7 nach Deitch (1955). Abweichend von der Originalvorschrift wurde 5 Std. lang gefärbt und exakt 5 Min. bei pH 2,7 gewässert.

Bei Zellen werden unspezifisch die basischen Gruppen überwiegend der Hexonbasen Lysin, Arginin und Histidin gefärbt. Die Reaktion hat sich als zuverlässiges Maß für den Gesamtproteingehalt von Zellkern und Cytoplasma erwiesen (Lederer u. Mitarb. 1966, Kraus u. Mitarb. 1968, Übersicht: Deitch, 1966). Am Bindegewebe haben Messungen der Azidophilie aus gleichen Gründen wie bei GC-Färbungen nur orientierenden Charakter (Kelly 1966).

c) *Millon*-Reaktion nach Rasch u. Mitarb. (1960).

Bei 496 nm werden spezifisch die Tyrosin-Gruppen erfaßt. Die Farbstoffkonzentration ist bei Zellen ein Maß für den Gesamtproteingehalt, bei Stützgeweben sind nur Objekte verwandter chemischer Zusammensetzung vergleichbar (Deitch, 1966).

Parallel-Schnitte wurden mit Hämatoxylin-Eosin gefärbt.

GERÄTE UND MESSUNGEN

Das Cytophotometer wurde aus im Handel befindlichen Teilen in einer Modifikation des von Sandritter u. Mitarb. (1959) beschriebenen Typs gebaut. Es besteht aus der Hochleistungslampe (12 V, 100 W), Leuchtfeld- und Meßfeldblende entsprechend 20 µ bzw. 0,7 µ Durchmesser in der Objektebene, achromatischem Kondensor der n. A. von rd. 0,3, Objektiv der n. A. 1,30 (Ölimmersion) bei Justierung nach dem Köhler'schen Prinzip, dem Sekundärelektronenvervielfacher (Typ 1P21, Fa. RCA), Spektralphotometer (Typ PMQ 2, Fa. Zeiss) und dem Netzgerät. Extinktionsmessungen erfolgten nach GC-Färbungen bei 573 nm (T_{max} 19%, HW 10 nm, Präzisionsinterferenz-Filter), nach NGS-Färbungen bei 483 nm (T_{max} 17%, HW 11 nm) und nach Millon-Färbungen bei 496 nm (T_{max} 19%, HW 9 nm).

Für Messungen des Eiweiß- und RNA-Gehaltes von Zellen dienten drei neugeborene und drei reife Tiere mit kurz entkalkten Felsenbeinen mit jeweils 4 bis 5 histologischen Schnitten pro Farbreaktion. Da neben der Farbstoffkonzentration die schwierig zu messende Schnittdicke (Weisbach, 1960, Übersichten: Sandritter 1964, Sandritter u. Mitarb., 1965) in den Extinktionswert eingeht, wurde die Schnittdickenbestimmung wie folgt umgangen.

2 Material und Methoden

VERSUCHSTIERE, HISTOLOGIE

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Die Schnitte wurden standardisiert in Xylol entparaffiniert, in *absolutem Äthanol gespült,* kurz luftgetrocknet und für die Interferometrie in Öl vom Brechungsindex n_{D} = 1 40 (Fa. Cargille New York) und 1 45 (geschliffene Objektträger Deckgläschen von 0 18 mm ± 10 % Dicke) für uv-mikrospektrographische Messungen in wasserfreiem Glyzerin n_{D} = 1 45 Deckgläser und Objektträger aus Quarz) nach Färbungen in Öl von angeglichenem Brechungsindex *(n_D = 1 52–1,53) eingedeckt*

FÄRBUNGEN

Zum photometrischen Nachweis chemischer Gewebsbestandteile wurden *folgende Farbreaktionen verwendet (Tab. I)*:

a) *Gallocyanin-Chromalaun* Färbung (GC) bei pH 1 64 nach Sandritter u. Mitarb. (1963 1966). Zur Kontrolle wurden Schnitte bei 50 C 2 Std. mit 0,5 % RNAse (Worthington) in A. dest. mit 0,2 *n* NaOH auf pH 7 0 eingestellt, *inkubiert und nach kurzem Spülen in A.* dest. gleichartig gefärbt.

Bei Zellen gilt die Reaktion als spezifisch für die Phosphatgruppen der DNS die stöchiometrisch erfaßt *wird (Sandritter u. Mitarb.,* 1966) und der cytoplasmatischen RNA (Mundkur 1961 Pakkenberg, *1962 Kiefer u* Mitarb., 1966)

versal-Mikrospektrographen (Typ UMSP I, Fa. Zeiss) unter folgenden Bedingungen.

Kondensor der n. A. 0,8 abgeblendet auf etwa 0,3 des Objektives 1,25 Meßfeldblende und Leuchtfeldblende von 0,5 μ bzw 8 μ Durchmesser in der Objektebene, Vorschub des Schreibers „20" (Einzelheiten bei Trapp 1966). An Schnecken wurden an 6–8 histologischen Schnitten 160 an Achillessehnen 20 kontinuierliche Absorptionsspektren zwischen 350 und 250 nm aufgenommen, bei 16 verschiedenen Wellenlängen in Extinktionswerte umgerechnet und zur Streulichtkorrektur nach Schramm u. Mitarb. (1944) im doppelt-logarithmischen Raster (Abb. 6) aufgezeichnet. Die Steigung der Streulicht Korrekturlinie ist dabei durch 4–6 Meßwerte im Wellenlängenbereich zwischen 350 und 310 nm gegeben. Es wurden die Relationen innerhalb der einzelnen Windungen bestimmt; zum Vergleich zwischen Ohren neugeborener und reifer Tiere mit Achillessehne wurden sämtliche Extinktionswerte nach Streulichtkorrektur gemittelt. Das Extinktionsmaximum wurde aus den Extinktionskurven abgelesen.

STATISTISCHE SICHERUNG

Die Unterschiede der Mittelwerte aller Messungen wurden statistisch nach dem *t* Test (Student, 1908) unter der Annahme einer Normalverteilung auch für interferometrische Dichtewerte geprüft. Als Grenzwert der Wahrscheinlichkeit wurde ein P Wert von 0,05 angenommen. Zur Berechnung wurden die Dichtewerte in g/cm bei UV-Messungen sowohl Einzelmessungen (Extinktionswerte) wie auch die in Prozent ausgedrückten Relationen innerhalb der Schneckenwindungen, bei cytophotometrischen Messungen der Farbstoffkonzentration nur die Relationen innerhalb der Windungsquerschnitte verwendet.

Das Gerät wurde vom Direktor des Instituts für Strahlenbiologie der Universität Münster zur Verfügung gestellt. Der Autor ist Herrn Prof. Dr. W. Dittrich auch für Kritik am Manuskript zu Dank verpflichtet.

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FEHLERDISKUSSION

Um Meßwerte der relativen Eiweiß- und RNA Konzentration im Cytoplasma von Zellen des Innenohres mit autoradiographischen Bestimmungen des Aminosäure und RNA Stoffwechsels (Meyer zum Gottesberge, 1961 et al.) vergleichen zu können, mußten entkalkte Felsenbeine benutzt werden. Bei kurzer Einwirkung von Formalin-Trichloressigsäure ist der Aktivitätsverlust inkorporierter Aminosäuren für zahlreiche Zelltypen gering und prozentual gleich (Antoni u. Mitarb., 1965 vgl. Ostrowski u. Mitarb., 1961 Lederer u. Mitarb. 1966 Kraus u. Mitarb., 1968 *b*). Auch am Innenohr sind Aktivitätsverluste (Koburg, 1961) und damit Verluste von cytoplasmatischen Proteinen zu vernachlässigen. Wie weit die Entkalkung zu RNA Verlusten führt, ist nicht genau abzugrenzen. ³H-Cytidinaktivitäten werden durch Säuren stärker reduziert (m-RNA? t-RNA? Schneider u. Mitarb. 1963 Übersicht: Schultze 1968) die Relationen der Silberkorndichte bleiben jedoch nach Entkalkung des Hörorganes erhalten (Koburg, 1961) so daß ein prozentual gleichmäßiger RNA Verlust anzunehmen ist.

Für quantitativ-histochemische Untersuchungen am membranösen Labyrinth wurden die Schnecken länger entkalkt, unterschiedlich hohe Substanzverluste einzelner Strukturen sind zu beachten.

Bei cytophotometrischen Messungen im sichtbaren und UV Bereich sind die apparativ bedingten Fehler zu vernachlässigen (rd. 5% Kondensorapertur Streulicht, Blendendurchmesser Schwarzschild-Villiger Effekt, Nicht Linearität, Spannungsschwankungen ausführliche Diskussion. Sandritter 1958, 1964 Sandritter u. Mitarb., 1965).

Als objektbedingte Fehler kommen Lipofuszin-Pigment der Haarzellen (Ishii u. Mitarb., 1967) in Frage. Striaepithelien mit stärkerem Melaningehalt wurden von den Messungen ausgeschlossen. Böttcher'sche Zellen konnten nicht immer sicher von Sulcusepithelien (zu niedrige Extinktionen), Zellen der Reissner'

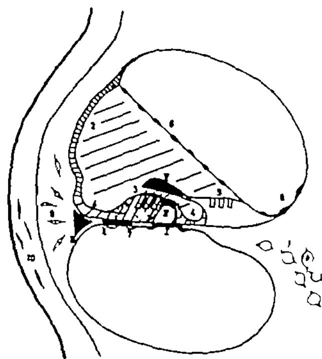


Abb 1 Schematische Darstellung einer Schneckenwindung vom Meerschweinchen mit untersuchten Zellen *1* Ganglienzellen des *Ggl spirale* *2* Epithel der *Stria vascularis*, *3* Corti'sches Organ (innere und äußere Haarzellen *a*, Deiters'sche Zellen *b* Hensen'sche Zellen *c* Böttcher'sche Zellen *4* inneres und äußeres *Sulcusepithel* *5* *Epithel des limbus spiralis*; *6* Zellen der Reissner'schen Membran *7* tympanale Belegzellen *8* Zellen am Boden der Scala vestibuli *9* Fibrozyten des *lig spirale* *10* Osteozyten der Schneckenkapsel. Schwarz Meßpunkte am membranösen Labyrinth. *Pars pectinata* (I) und *tecta* (II) der BM *lig spirale* (III), Pfeilerkopf (IV) und Tectorialmembran (V) Endolymphe schraffiert.

Innerhalb einer Schneckenwindung wurden 6 bis 10 Ganglienzellen und Striaepithelien vom großen und kleinen bzw chromophoben und chromophilen Typ alle Zellen des Corti schen Organes und je 5 übrige Zellen (Abb 1) mit 10 einzelnen Extinktionsbestimmungen pro Zelle untersucht. Die Extinktionswerte vom Cytoplasma wurden für jeden Zelltyp gemittelt, die Mittelwerte in Prozent Werte umgerechnet, wobei die Meßwerte von Ganglienzellen (= 100%) als Bezugswert dienten. Auf diese Weise wurden pro Farbreaktion an 25 bis 30 Schneckenwindungen die mittleren Relationen des cytoplastmatischen Eiweiß- und RNA-Gehaltes bestimmt. Für Messungen der Eiweißkonzentration und GC Färbbarkeit am membranösen Labyrinth wurde gleichartig verfahren.

Für Dichtebestimmungen am Durchlicht Interferenzmikroskop nach Jamin Lebedeff (Typ Standard WL, Fa. Zeiss) wurden folgende Bedingungen eingehalten Kondensor der n. A. etwa 0,3 des Objektives 0 65 Gangunterschiedsbestimmungen erfolgten bei 546,1 nm mit der subjektiven Analysatormethode im homogenen Feld nach Dunkeladaptation (Übersicht: Beneke, 1966) Zur Eliminierung der Schnittdicke (Pehland u Mitarb., 1959) wurde der Gangunterschied zuerst in Öl vom Brechungsindex $n_{D\infty} = 1\,40$ gemessen der Meßpunkt von 2–3 μ Durchmesser in einer Skizze genau markiert, das Präparat in Öl von $n_{D\infty} = 1\,45$ eingedeckt und die Gangunterschiedsbestimmung wiederholt. Die Dichte (c) wurde nach der Formel

$$c = \frac{L_1(n_{m2} - n_e) - L_2(n_{m1} - n_e)}{x(L_1 - L_2)} \qquad (g\,cm^{-3})$$

berechnet wobei L_1 und L_2 die gemessenen Gangunterschiede bzw Phasenverschiebungen n_{m1} und n_{m2} den Brechungsindex der Eindeckmittel n_e den Brechungsindex von A dest. (1 333) und x das spezifische Refraktionsinkrement darstellen (0 0018 vgl Davies, 1958) Von jeweils drei neugeborenen, drei senilen und 4 reifen Tieren wurden 5 bis 6 histologische Schnitte verwendet. Die Meßpunkte sind die gleichen wie nach Bestimmungen der Farbstoffkonzentration (Abb 1) Reifung und Alterung des membranösen Labyrinthes wurden nur an der Basilarmembran verfolgt (Tab I).

Zur Kontrolle der Interferometrie wurden aus 12 Std. fixierten Schnecken Häutchen von *stria vascularis* und BM (Neubert, 1950 Beck, 1964) in gestuft konzentrierten Mischungen von Glucose, NaCl und Glyzerin mit A dest. suspendiert und bei dem Dichtewert abgelesen bei dem das Häutchen in der Schwebe blieb (vgl Bürger 1961) Die Meßergebnisse waren nicht reproduzierbar

Absorptionsmessungen im UV erfolgten an 2 neugeborenen und 2 reifen Tieren (Meßpunkte vgl. Abb

3 Ergebnisse

MORPHOLOGIE

Die äußerst empfindlichen Zellen des Corti'schen Organes zeigen bei kurzen Entkalkungszeiten morphologisch weitgehende Integrität (Abb 2) Die Tectorialmembran ist wie nach Entkalkungen üblich von der *lamina reticularis* abgerissen, Zellen und häutiges Labyrinth erscheinen nach NGS und GC Färbungen je nach Farbstoffkonzentration im Hell-Dunkel-Kontrast. Knochengewebe weist nach GC Färbungen im polychromatischen Licht einen violetten Farbton auf (Pseudometachromasie), der am membranösen Labyrinth nur angedeutet ist.

Nach längerer Entkalkung für Untersuchungen am membranösen Labyrinth finden sich an Tectorialmembran und den Zellen des Innenohres interferenzmikroskopisch (Abb. 3) und bei Beobachtung im UV Licht (Abb 4) Auflösungserscheinungen. Doch ist das Stützgewebe von den Zellen gut abzugrenzen.

ERGEBNIS DER MESSUNGEN AN ZELLEN

Als Maß für die relative Eiweiß- und RNA Konzentration im Cytoplasma von Zellen des Innenohres wurde cytophotometrisch die Konzentration von NGS Millon s Reagens und GC bestimmt (Tab. II). Die höchste Färbbarkeit weisen Ganglienzellen des *ggl. spirale* auf es folgen Striaepithelien mit Osteo- und Fibrozyten, während Zellen der Reissner'schen Membran, tympanale Belegzellen und Epithelien des *limbus spiralis* wesentlich niedriger einzuordnen sind. Sehr geringe Farbstoffkonzentrationen finden sich in den Zellen des Corti'schen Organes, wobei Sulcusepithelien, Hensen und Deiters'sche Zellen die niedrigste und innere Haarzellen eine deutlich höhere Farbstoffbindung aufweisen als äußere Haarzellen.

Für die einzelnen Zelltypen stimmen NGS- und Millon-Farbstoffkonzentration recht gut überein (Tab. II, Spalte 1 und 2) unterschiedlich verhält sich der Quotient aus NGS- und GC-Farbstoffgehalt (Spalte 4) Für fast alle untersuchten Zelltypen liegt dieser Wert mit 1 0 bis 1,3 gleich hoch er steigt für Zellen des Corti'schen Organes auf das 1,5 bis 2,5-fache an.

DISKUSSION

Mit quantitativ-histochemischen Methoden wurde bisher nur der Einfluß von Schall auf die RNA und Proteinkonzentration der Ganglienzellen des *ggl. spirale* verfolgt (Hamberger u Mitarb 1945 Hammer 1956 Pakkenberg u. Mitarb., 1964) Vergleichende Untersuchungen an weiteren Zellen des Innenohres stehen aus. Die festgestellten Unterschiede der Farbstoffkonzentration stehen in guter Übereinstimmung mit der Ultrastruktur des membranösen Labyrinthes (Übersichten: Engström, 1965 Spoendlin, 1966 a Iurato 1967) Ganglienzellen und Striaepithelien weisen pro Volumeneinheit im Cytoplasma einen wesentlich höheren Gehalt an Ergastoplasma auf als Sulcusepithelien, und in inneren Haarzellen finden sich relativ mehr cytoplasmatische Organellen als in äußeren Haarzellen.

Ein Vergleich mit autoradiographischen Meßergebnissen des Aminosäure-Stoffwechsels zeigt, daß die Silberkorndichte (Meyer zum Got

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Obwohl die Empfindlichkeit der Methode $6\,6 \times 10^{-13}$ g/cm³ erreicht (Bartels, 1966) steigt der hohe Fehler interferometrischer Massenbestimmungen (rd 15% Kraus u Mitarb 1968) bei Dichtemessungen an (Pehland u Mitarb 1959) Dafür dürfte die Objektinhomogenität (Abb 3) und die Anisotropie des membranösen Labyrinthes (Iurato, 1960 1962) verantwortlich sein. Bei jeweils 70 Gangunterschiedsbestimmungen an BM und *Tectorialmembran nach Ausrichten* und im azimutalen Abstand von 90 (Gahm 1962, 1963) fand sich eine mittlere Abweichung von 4 9% die nach dem *mittleren Fehlerfortpflanzungsgesetz* nur zum Teil dem Gesamtfehler addiert werden kann. Ferner stellt das zur Dichteberechnung verwendete Refraktionsinkrement ($x=0\,0018$) einen Näherungswert dar (Benecke, 1966 vgl Buddecke u Mitarb 1967) Mögliche Änderungen während der Reifung oder durch die Fixierung (Hancox u Mitarb 1956 Kraus u Mitarb., 1968 *b*) müssen als Fehlerquelle hingenommen werden.

3 Ergebnisse

MORPHOLOGIE

Die äußerst empfindlichen Zellen des Corti'schen Organes zeigen bei kurzen Entkalkungszeiten morphologisch weitgehende Integrität (Abb. 2). Die Tectorialmembran ist wie nach Entkalkungen üblich von der *lamina reticularis* agerissen, Zellen und häutiges Labyrinth erscheinen nach NGS und GC Färbungen je nach Farbstoffkonzentration im Hell-Dunkel-Kontrast. Knochengewebe weist nach GC Färbungen im polychromatischen Licht einen violetten Farbton auf (Pseudometachromasie), der am membranösen Labyrinth nur angedeutet ist.

Nach längerer Entkalkung für Untersuchungen am membranösen Labyrinth finden sich an Tectorialmembran und den Zellen des Innenohres interferenzmikroskopisch (Abb 3) und bei Beobachtung im UV Licht (Abb. 4) Auflösungserscheinungen. Doch ist das Stützgewebe von den Zellen gut abzugrenzen.

ERGEBNIS DER MESSUNGEN AN ZELLEN

Als Maß für die relative Eiweiß- und RNA Konzentration im Cytoplasma von Zellen des Innenohres wurde cytophotometrisch die Konzentration von NGS, Millon s Reagens und GC bestimmt (Tab II). Die höchste Färbbarkeit weisen Ganglienzellen des *ggl. spirale* auf, es folgen Striaepithelien mit Osteo- und Fibrozyten, während Zellen der Reißner schen Membran, tympanale Belegzellen und Epithelien des *limbus spiralis* wesentlich niedriger einzuordnen sind. Sehr geringe Farbstoffkonzentrationen finden sich in den Zellen des Corti'schen Organes, wobei Sulcusepithelien, Hensen- und Deiters'sche Zellen die niedrigste und innere Haarzellen eine deutlich höhere Farbstoffbindung aufweisen als äußere Haarzellen.

Für die einzelnen Zelltypen stimmen NGS- und Millon-Farbstoffkonzentration recht gut überein (Tab II, Spalte 1 und 2) unterschiedlich verhält sich der Quotient aus NGS- und GC Farbstoffgehalt (Spalte 4) Für fast alle untersuchten Zelltypen liegt dieser Wert mit 1 0 bis 1,3 gleich hoch er *steigt für Zellen* des Corti'schen Organes auf das 1,5 bis 2,5-fache an.

DISKUSSION

Mit quantitativ-histochemischen Methoden wurde bisher nur der Einfluß von Schall auf die RNA- und Proteinkonzentration der Ganglienzellen des *ggl spirale* verfolgt (Hamberger u. Mitarb., 1945 Hammer 1956 Pakkenberg *u. Mitarb., 1964)* Vergleichende Untersuchungen an weiteren Zellen des Innenohres stehen aus. Die festgestellten Unterschiede der Farbstoffkonzentration stehen in guter Übereinstimmung mit der Ultrastruktur des membranösen Labyrinthes (Übersichten· Engström, 1965 Spoendlin, 1966 u Iurato, 1967). Ganglienzellen und Striaepithelien weisen pro Volumeneinheit im Cytoplasma einen wesentlich höheren Gehalt an Ergastoplasma auf als Sulcusepithelien, und in inneren Haarzellen finden sich relativ mehr cytoplasmatische Organellen als in äußeren Haarzellen.

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Obwohl die Empfindlichkeit der Methode $6\,6 \times 10^{-14}$ g/cm² erreicht (Bartels, 1966), steigt der hohe Fehler interferometrischer Massenbestimmungen (rd. 15% Kraus u Mitarb 1968) bei Dichtemessungen an (Pehland u *Mitarb 1959) Dafür dürfte die Objektinho*mogenität (Abb 3) und die Anisotropie des membranösen Labyrinthes (Iurato, 1960 1962) verantwortlich sein Bei jeweils 70 Gangunterschiedsbestimmungen an BM und Tectorialmembran nach Ausrichten und im azimutalen Abstand von 90° (Gahm 1962, 1963) fand sich eine mittlere Abweichung von 4 9% die nach dem mittleren Fehlerfortpflanzungsgesetz nur zum Teil dem Gesamtfehler addiert werden kann. Ferner stellt das zur Dichteberechnung verwendete Refraktionsinkrement ($x=0\,0018$) einen Näherungswert dar (Benecke, 1966 vgl Buddecke u Mitarb 1967) Mögliche Änderungen während der Reifung oder durch die Fixierung (Hancox u Mitarb 1956 Kraus u Mitarb., 1968 *b*) müssen *als Fehlerquelle hingenommen werden*



Abb. 3 Corti'sches Organ im Interferenzmikroskop bei 546 nm und maximaler Dunkelstellung der BM. Am rechten Bildrand das sogenannte Schattenbild. 600

noch aussteht (Ficqu u. Mitarb. Niklas u. Mitarb., 1956 Übersicht: Schultze, 1968). Nur Grasso u. Mitarb (1963 1966) berichten über mangelnde Parallelität zwischen Eiweißsynthese und cytoplasmatischer RNA Konzentration während der Reifung von Reticulocyten, die mit Verschiebungen der ribosomalen Aktivität verbunden ist (veränderte Halbwertszeiten der RNA Fraktionen? Fantoni u. Mitarb., 1968 Burka, 1968).

Während noch in der älteren biochemischen Literatur für Bakterien und Zellen eine unspezifische und einheitlich hohe ribosomale Aktivität angenommen wurde (Wilson u Mitarb., 1965 Übersichten: Petermann, 1964 Schweet u. Mitarb., 1966) stehen die an Zellen des Innenohres festgestellten Unterschiede in der Intensität der ribosomalen Peptidsynthese in guter Übereinstimmung mit neueren Erkenntnissen des cytoplasmatischen Eiweißstoffwechsels. Im Verlauf von Wachstum bzw Mitosezyklus (Lucas u. Mitarb., 1964 Salb u. Mitarb 1965 Sykes u Mitarb Enger u. Mitarb 1968 vgl Kiefer u. Mitarb., 1968), während der Embryogenese (Monroy u. Mitarb 1965) Reifung und Alterung (Yamagami u Mitarb 1966) und nach Hungeratrophie (v d. Decken, 1967) schwankt die vom einzelnen Ribosom in der Zeiteinheit synthetisierte Proteinmenge *in vitro* beträchtlich.

Für eine hohe Intensität der ribosomalen Funktion im Corti'schen Organ spricht das Vorkommen der Hensen-Körper in den äußeren Haarzellen, die in ihrer Ultrastruktur (Spoendlin, 1957 1966 a) den sogenannten Nebenkernen entsprechen und als morphologisches Äquivalent einer „intensiven Proteinsynthese gelten (Übersicht: Mölbert, 1968). Ferner stimmt die histochemisch bestimmte Aktivität der Monoaminooxydase (Schätzle u. Mitarb 1967), die allerdings keinen sicheren Indikator der Ribosomenfunktion darstellt, mit der ribosomalen Syntheserate relativ gut überein. Als Ursache für die unterschiedlich intensive Peptidsynthese in den Zellen des Innenohres sind cytoplasmatische Faktoren zu vermuten, da historadiographisch mit Ausnahme von Zellen des *ggl spirale* zwischen RNA Umsatz und Aminosäure-Einbaurate als Maß für die genetische Aktivität Parallelität besteht (Koburg, 1961)

Zu beachten ist die mitochondriale Eiweißsynthese. Bei hohem Cytidinumsatz der mitochondrialen DNS (Chang u. Mitarb 1968) ist wegen der vergleichsweise niedrigen RNA Konzentration (Siebert, 1968 vgl. Tyler 1967)



Abb 2 Histologischer Schnitt eines Corti'schen Organes nach Färbung der Gesamtproteine mit NGS, aufgenommen bei 483 mm, lin. Vergr 900

tesberge 1961 Meyer zum Gottesberge u Mitarb 1965 vgl Koburg u Mitarb., 1962 Plester u Mitarb 1962 Schreiner 1965) nicht mit der cytoplasmatischen RNA Konzentration der Zellen des Innenohres (Tab II Spalte 3 5 und 6) korreliert ist Zellen des Corti schen Organes weisen im Vergleich zu Ganglienzellen einen bis zu siebenfach üb r höhten Eiweißumsatz bezogen auf die Färbbarkeit des Cytoplasma mit GC auf Da mit der GC Färbung die cytoplasmatische RNA Konzentration erfaßt wird die zu 80% oder mehr (Watson 1965 Siebert 1968) aus r RNA besteht, sind die Extinktionswerte ein relatives Maß für die Konzentration von r RNA im Cytoplasma, und der Quotient aus Aminosäure Inkorporation und RNA Konzentration im Cytoplasma bezeichnet die mittlere Aktivität der Ribosomen bei der Eiweißsynthese Nach den Meßergebnissen zu urteilen liegt sie bei Ganglienzellen und Striaepithelien in der gleichen Größenordnung wie bei Fibrozyten tympanalen Belegzellen und Zellen der Reissner schen Membran. Äußere Haarzellen weisen eine intensivere *Proteinsynthese auf als innere Haar*zellen die höchsten Aktivitäten sind dem Sulcusepithel und den Stützzellen der Rezeptoren *zuzuschreiben* Demnach wäre die Intensität der ribosomalen Eiweißsynthese im Cytoplasma eine zellspezifische Größe

In Anbetracht der Fehlermöglichkeiten quantitativ-histochemischer Methoden (vgl. Fehlerdiskussion) ist zu fragen ob Systemfehler für die Meßergebnisse verantwortlich sind Säurebehandlung scheint unwesentlich zu sein An unentkalkten in Kunststoffen eingebetteten Schnecken ist gleichfalls eine fehlende Parallelität zwischen cytoplasmatischer relativer RNA Konzentration und der Silberkorndichte nach Gabe von ³H Leucin (Kraus u. Richrath unpubliziert) festzustellen Fehlende Spezifität bei der Anfärbung der ribosomalen Phosphatgruppen ist unwahrscheinlich, jedoch nicht mit letzter Sicherheit auszuschließen Nicht Linearität zwischen Farbstoffkonzentration und Extinktionswerten ist zumindest für Zellen mit 20% und mehr des RNA-Gehaltes von Ganglienzellen kaum anzunehmen die gemessenen Extinktionen liegen mit 0 2 bis 0 8 im optimalen *Bereich des photometrischen* Nachweises.

Vergleichsmöglichkeiten mit anderen Orga*nen sind nicht gegeben* da eine Korrelation der cytoplasmatischen Basophilie mit den zahlreichen autoradiographischen Messungen des Eiweißstoffwechsels verschiedener Zelltypen

ter der Plasmamembran mit angrenzender Corti-Lymphe ausgerichtet ist. Bekannt ist, daß Intensität und Genauigkeit der Translation in zellfreien Systemen von der Kationenkonzentration (Davies u. Mitarb. Szer u. Mitarb., Friedman u. Mitarb. So u. Mitarb., 1964 Shaeffer u. Mitarb. 1968), dem pH Wert (Grunberg u. Mitarb. 1965), der Temperatur (Szer u. Mitarb. Friedman u. Mitarb. 1964 McCormick u. Mitarb. 1968) und dem Aminosäurespiegel (Baliga u. Mitarb. Sarkar u. Mitarb. 1968) abhängen. Wie weit diese Parameter des „coding environment" am Innenohr von Einfluß sind, ist nicht zu entscheiden, da synoptische vergleichende Untersuchungen an Zellen des Corti'schen Organes und den verschiedenen Lymphen fehlen. Bei den relativ kleinen Kontaktflächen der Zellen des Corti'schen Organes ist auch an das Phänomen der Kontaktinhibition mit Verschiebung der prozentualen Synthese von t m- und r RNA (Rhode u. Mitarb., 1968) zu denken.

Als kausaler Faktor der weitgehend selektiven Ototoxizität der Antibiotica der Streptomycin-Gruppe wurde eine Kumulierung in den Lymphen des Innenohres angenommen (Velikorussova, 1964 Muraveiska, 1965 Stupp u. Mitarb. 1966 Stupp 1969) Doch betragen z. Bsp. die gemessenen Neomycin-Konzentrationen höchstens 20% der maximalen Serumkonzentration (Voldřich, 1965) bei teilweise exzessiv hohen Konzentrationen in Urin und Galle fehlt eine entsprechende Organotropie (Übersichten. Weinstein, 1965 Walter u. Mitarb., 1965) Andererseits sind trotz niedriger Konzentrationen des Antibioticums im Gehirn (André, 1956 Stupp u. Mitarb. 1966 1969) Schädigungen an N. opticus und ZNS relativ häufig. Es liegt nahe, als prädisponierenden Faktor die im Vergleich zu Ganglienzellen hohe ribosomale Syntheserate des Corti'schen Organes anzusehen. Äußere Haarzellen, die gegenüber Streptomycin am empfindlichsten sind (Beck u. Mitarb. 1962 Farkashidy u. Mitarb., 1963 Hawkins u. Mitarb. 1964 Spoendlin, 1966 *b* Übersicht: Engström u. Mitarb., 1965), zeigen eine intensivere Eiweißsynthese

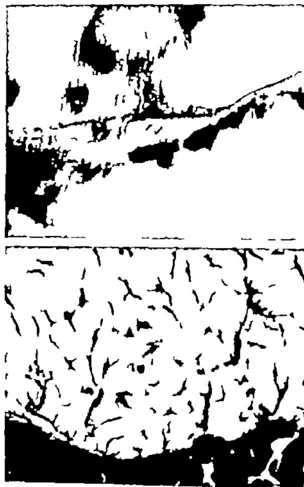


Abb. 4. UV-Mikroskopie bei 270 nm. Oben: *pars recta* der BM innere Haarzelle außerhalb des Focus (1 00). Unten: Achillessehne vom reifen Meerschweinchen, Muskelzellen und Zellkerne von *Fibrozyten* dunkel, Sehnengewebe hell (460).

als innere Haarzellen. Über Streptomycinschäden von *Stützzellen* und Sulcusepithelien, die die höchste Syntheserate aufweisen, wird aber nur vereinzelt berichtet (Reddy u. Mitarb., 1962 Hawkins u. Mitarb., 1964) Sicher sind quantitative Gesichtspunkte allein nicht maßgebend.

Streptomycin blockiert die ribosomale Eiweißsynthese. Es führt zu Ablesefehlern mit Einbau von falschen Aminosäuren (Davies u. Mitarb., 1964). Neben den oben angeführten Faktoren des „coding environment" hängt das Ausmaß des Ablesefehlers auch von der Ba-

Tab II *Relationen der cytophotometrisch gemessenen Eiweißkonzentration nach NGS Färbung (Spalte 1) und Millon-Färbung (Spalte 2) und RNA Konzentration nach GC Färbung (Spalte 3) im Cytoplasma von Zellen des Innenohres vom Meerschweinchen Spalte 4 Quotient aus Eiweiß- und RNA Konzentration Spalte 5 Historadiographisch gemessene Eiweiß-Umsatzrate (Mittelwerte aus: Meyer zum Gottesberge 1961 und Koburg u Mitarb 1962 a) Spalte 6 Quotient aus Eiweiß-Umsatzrate und RNA Konzentration*

Angaben in Prozent. Bezugswert Ganglienzellen des *ggl spirale* = 100 %, p = Statistik.

	1 Eiweißgehalt (NGS)	*p*	2 Eiweißgehalt (Millon)	*p*	3 RNA-Gehalt (Gallocyanin-Chromalaun)	*p*	4 Eiweißgehalt pro RNA Gehalt (NGS/GC)	5 Eiweiß-Umsatz (H Leucin, ³H Lysin)	6 Eiweiß-Umsatz pro RNA-Gehalt
Ganglienzellen (*ggl spirale*)	100		100		100		1,0	100	1 0
		0,001		0,001		0,001			
Striaepithel	79		74		59		1,3	71	1,2
		0 001		0,001		0 001			
Haarzellen	29		3[illegible]		19		1,5	53	2,8
Äußere Hz.	26		30		17		1,5	53	3,1
		0,15		0,3		0,003			
Innere Hz.	31		35		21		1,5	53	2,5
Deiters'sche Zellen	20				12		1 7	50	4,2
Hensen'sche Zellen	22	Hz. 0 01			9	Hz. 0,001	2,5	50	5 6
Böttcher'sche Zellen	25				11		[illegible]3	81	7 4
		0,008 Hz. 0,7				0,001 Hz. 0,01			
Sulcusepithel	28				15		1 9	42	2,8
Limbusepithel	53				38		1 4	35	0,9
Zellen der Reissner'schen Membran	30	Hz. 0,05			40	Hz. 0,01	0,8	53	1,3
Zellen der *scala vestibuli*	45				34		1,3	58	1 7
		0 15				0,001			
Tympanale Belegzellen	55				65		0,9	54	0,8
Fibrozyten, *lig spirale*	74				65		1 1	68	1 1
		0,3				0,001			
Osteozyten, Schneckenkapsel	82				80		1,0	38	0,5

die mitochondriale Proteinsynthese jedoch quantitativ zu vernachlässigen

Bei Zellen mit hoher ribosomaler Aktivität wurde cytophotometrisch ein niedriger Gehalt an RNA im Vergleich zu den Gesamtproteinen gemessen. Offensichtlich überwiegen nach elektronenmikroskopischen Beobachtungen in Ganglienzellen und Striaepithelien das geordnete und rauhe endoplasmatische Reticulum (*Iurato 1967 Rosenbluth 1967*) und in Sulcusepithelien und äußeren Haarzellen die freien Polysomen (*Spoendlin, 1966 a*) Wie weit freie Polysomen den ribosomalen Funktionszyklus (Schlessinger u. Mitarb., *1967* Kaempfer 1968) schneller durchlaufen ist nicht zu entscheiden, da biochemische Angaben über Aktivitätsunterschiede zwischen beiden Systemen außerordentlich widersprüchlich sind (Campbell u Mitarb., 1964 Hallinen u. Mitarb Manganiello u Mitarb O'Neal u Mitarb 1965 Dallner u Mitarb Seekely u. Mitarb Kuff u Mitarb 1966 Bloemendal u Mitarb Eliasson u Mitarb., 1967 Schreml u. Mitarb., 1968)

Auffällig ist daß in äußeren Haarzellen ein Teil des Ergastoplasma linear unmittelbar un-

Tab IV *Dichte (g/cm³) und Relationen der mittleren UV Extinktion und Farbstoffkonzentration von Millon s Reagens, NGS und GC der Basilarmembran in verschieden hohen Windungen.*

Bezugswert, BM der untersten Windung 100 %. Im Vergleich zur untersten Windung signif kante Mittelwerte kursiv (P 0,05)

	Dichte in g/cm³		Extinktion in Prozent (reife Tiere)			
	Neugeborene Tiere	Senile Tiere	UV	Millon	Naphtolgelb S	Gallocyaninchromalaun
Basal			100	100	100	100
	1,88	1,34				
Mitte			87	*86*	107	*111*
	1,72	1,29				
Apikal			110	*81*	95	*123*

Dichtewerte die für alle untersuchten Objekte gleich ist. Die Kurve (Abb 5) ist infolge einer relativen Häufung höherer Meßwerte asymmetrisch. Nach Auftragen im Wahrscheinlichkeitsnetz erweist sich die Verteilung als normal-logarithmisch.

Ultraviolett-mikrospektrographisch bleibt während der Reifung die mittlere, streulichtkorrigierte Extinktion aller Strukturen des membranösen Labyrinthes unverändert (Abb 6) Sie ist mit 0,29 für neugeborene ($n = 70$ $s = 0{,}08$) und reife Tiere gleich hoch ($n = 70$ $s = 0{,}06$). Das Absorptionsmaximum verschiebt sich während des Wachstums von 270 nm bei neugeborenen ($s = 3{,}8$) nach 277 nm bei reifen Tieren ($s = 1{,}9$ $P = 0{,}001$). Gleichzeitig nimmt der Streulichtanteil an der Gesamtabsorption im Absorptionsmaximum ab die Steigung der Korrekturgeraden sinkt von 23 7 ($\mathrm{tg}\,\alpha = 0{,}38$ $s = 12{,}3$) auf 18 8 ($\mathrm{tg}\,\alpha = 0{,}32$ $s = 9{,}0$ $P = 0{,}01$).

An der Achillessehne ist die mittlere Extinktion mit 0,05 ($n = 20$ $s = 0{,}01$ $P = 0{,}001$) wesentlich niedriger Die Steigung der Streulicht Korrekturgeraden liegt bei 12,4 ($\mathrm{tg}\,\alpha = 0{,}22$ $P = 0{,}005$) das Absorptionsmaximum ist nur angedeutet bei 275 nm zu erkennen.

DISKUSSION

Die Dichte morphologischer Strukturen im histologischen Schnitt ist eine Resultante aus dem Verhältnis von Fibrillen zu Grundsubstanz, deren chemischer Zusammensetzung und Hydratationsgrad *in vivo* der von der Molekularstruktur dem Grad der Polymerisation und der Vernetzung der Makromoleküle (Wasserstoffbrückenbindungen, van der Waal'sche Kräfte, elektrostatische Wechselbindungen) und der histologischen Aufarbeitung abhängt. Bei isoliertem Kollagen kann die Dichte je nach Fraktion und Restfeuchtigkeit zwischen 1,34 g/cm³ (Fessler u. Mitarb., 1962) und 1,56 g/cm³ (Kanagy u. Mitarb 1943) betragen, während Keratine (1,3 g/cm³ Finean, 1967), Gelatine (1,27 g/cm³ Hodgman, 1946 1,35 g/cm³ Cowan u Mitarb., 1953) und Fibrinogen (1 39 g/cm³ Scheraga u. Mitarb., 1954) eine niedrigere Substanzkonzentration aufweisen Auch mit Angaben über die Dichte von Zellbestandteilen besteht gute Übereinstimmung (isolierte Zellkerne: 1,3 g/cm³ Behrens, 1956 Ribosomen. 1,6 g/cm³ Sanchez de Jimenez u. Mitarb., 1968 Viren 1,33 g/cm³ Bawden u. Mitarb., 1937 DNS 1 7 g/cm³ Arrhigi u. Mitarb., 1968).

Mit gleicher Methodik wurde an Sehnenfäden der Mitralis eine Substanzkonzentration von 1 15 g/cm³ gemessen (Beneke u. Mitarb., 1967 vgl. Bürger 1961). Demnach muß die BM sehr festes Sehnengewebe darstellen. Von großem Interesse für die Schwingungsgleichung des Innenohres (Impedanz der BM, Schallschnelle, Schallgeschwindigkeit, vgl. Ranke, 1942, 1950 Zwislocki, 1948 Übersicht: Keidel, 1965) wären Dichtewerte der BM *in vivo*

Tab III *Relationen der mittleren UV Extinktionen und der Farbstoffkonzentration von Millons Reagens NGS und GC des membranösen Labyrinthes*

Bezugswert *Pars tecta* der BM = 100 Im Vergleich zur BM signifikante Mittelwerte kursiv ($P < 0{,}05$)

	UV	Millon	Naphtolgelb S	Gallocyanin-Chromalaun
Basilarmembran				
pars pectinata	100	100	100	100
Lig spirale	—	*59*	*74*	78
Pfeilerkopf	*166*	*235*	*160*	1 4
Tectorialmembran	*74*	*51*	*27*	*139*

senzusammensetzung der m-RNA ab (Übersicht: Gorini u Mitarb 1968) Bei *e coli* entscheidet die genabhängige Molekularstruktur von Proteinen des 30 S Ribosoms über die *Streptomycinempfindlichkeit innerhalb* von zwei Zehnerpotenzen („core particle" Leboy u Mitarb., 1964 Staehelin u Mitarb 1966) Ferner soll Streptomycin die Kopplung von t RNA an das Ribosom beeinflussen (Kaji u Mitarb 1965) und die Dissoziation des 70 S Ribosoms behindern (Herzog, 1964 Leon u. *Mitarb 1967 Wolfe u Mitarb., 1968)* Über die m RNA oder Molekularstruktur der ribosomalen Eiweißkörper der Zellen des Hörorganes ist z. Zt. nichts bekannt Doch wird neuerdings über immunologisch faßbare Organspezifität der ribosomalen Proteine berichtet (Bielka u Mitarb 1968) die möglicher*weise für die Ototoxizität des Streptomycins* verantwortlich ist. Auch ist an Störungen der Membranpermeabilität durch dieses Antibioticum zu denken. Bei *e coli* sind auch sie mit der Streptomycinempfindlichkeit des Eiweißstoffwechsels korreliert (Tamás u Mitarb., 1968)

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Cytophotometrische Messungen (Tab III) zeigen eine starke Färbbarkeit des Pfeilerkopfes mit NGS Millons Reagens und hohe UV Extinktionen im Vergleich zur BM während an der Tectorialmembran relativ die Farbstoffkonzentration von GC überwiegt.

In den unteren Windungen ist die BM bei neugeborenen um 0 16 g/cm³ (*n* = 240 *P* = 0 02) und bei senilen Tieren um 0 05 g/cm³ (*n* = 240 *P* = 0 2) dichter als in den Windungen der oberen Schneckenhälfte. Gleichzeitig findet sich basal eine relativ *starke Färb*barkeit mit Millons Reagens bei abgeschwächter Färbbarkeit mit GC (Tab IV) UV Extinktionen und Gehalt an NGS sind praktisch gleich

Sehr kompakt ist die BM bei neugeborenen Tieren (1 76 g/cm *n* = 450 *s* = 0,57) Die Dichte nimmt bis zur Geschlechtsreife stark bis auf 1 33 g/cm³ (*n* = 130 *s* = 0,28 *P* = 0 001) ab sie wird während des Alterns geringer reduziert (1 29 g/cm³ *n* = 390 *s* = 0 39 *P* = 0 08)

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Bezugswert, BM der untersten Windung 100 %. Im Vergleich zur untersten Windung signifikante Mittelwerte kursiv (P 0,05)

	Dichte in g/cm³		Extinktion in Prozent (reife Tiere)			
	Neugeborene Tiere	Senile Tiere	UV	Millon	Naphtolgelb S	Gallocyaninchromalaun
Basal			100	100	100	100
	1,[illegible]	1,34				
Mitte			87	86	107	*111*
	1,72	1,29				
Apikal			110	*81*	95	*123*

Dichtewerte, die für alle untersuchten Objekte gleich ist. Die Kurve (Abb 5) ist infolge einer relativen *Häufung* höherer Meßwerte asymmetrisch. Nach Auftragen im Wahrscheinlichkeitsnetz erweist sich die Verteilung als normal-logarithmisch.

Ultraviolett-mikrospektrographisch bleibt während der Reifung die mittlere, streulicht korrigierte Extinktion aller Strukturen des membranösen Labyrinthes unverändert (Abb 6) Sie ist mit 0,29 für neugeborene ($n = 70$ $s = 0{,}08$) und reife Tiere gleich hoch ($n = 70$, $s = 0{,}06$). Das Absorptionsmaximum verschiebt sich während des Wachstums von 270 nm bei neugeborenen ($s = 3{,}8$) nach 277 nm bei reifen Tieren ($s = 1{,}9$; $P = 0{,}001$). Gleichzeitig nimmt der Streulichtanteil an der Gesamtabsorption im Absorptionsmaximum ab die Steigung der Korrekturgeraden sinkt von 23 7 ($\operatorname{tg} \alpha = 0{,}38$ $s = 12{,}3°$) auf 18,8 ($\operatorname{tg} \alpha = 0{,}32$ $s = 9{,}0$ $P = 0{,}01$).

An der Achillessehne ist die mittlere Extinktion mit 0,05 ($n = 20$; $s = 0{,}01$ $P = 0{,}001$) wesentlich niedriger Die Steigung der Streulicht-Korrekturgeraden liegt bei 12,4 ($\operatorname{tg} \alpha = 0{,}22$ $P = 0{,}005$) das Absorptionsmaximum ist nur angedeutet bei 275 nm zu erkennen.

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	Neugeborene Tiere	Senile Tiere	UV	Millon	Naphtolgelb S	Gallocyaninchromalaun
Basal			100	100	100	100
	1,88	1,34				
Mitte			87	86	107	*111*
	1 72	1,29				
Apikal			110	*81*	95	*123*

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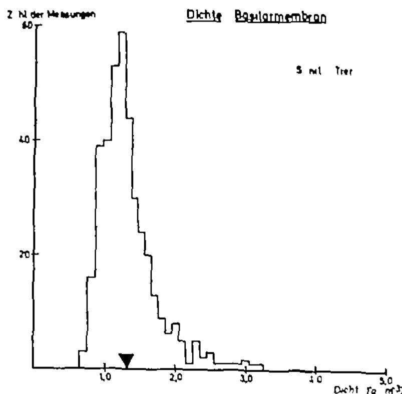


Abb 5 Häufigkeitsverteilung der Dichtewerte von der BM bei senilen Meerschweinchen. Abszisse Dichte in g cm Ordinate: Zahl der Messungen.

Sie können aus unseren Meßwerten nur angenähert unter Annahme eines Wassergehaltes von 70–80 Vol.% und von maximalem Wasserentzug und Schrumpfung bei der histologischen Aufarbeitung mit 1 15–1 23 g/cm³ für neugeborene und 1 07–1 10 g/cm³ für reife Tiere beziffert werden. Dabei handelt es sich wegen wahrscheinlicher Substanzverluste bei der Entkalkung um Mindestwerte

Die hohe Substanzkonzentration der BM im Vergleich zum spezifischen Gewicht von Lymphen bei Säugern (1 02–1 03 g/cm³ v Békésy 1960 vgl Rauch, 1964) ist mit der beobachteten synchronen Schwingungsform von Reissner scher Membran, Endolymphe und BM (v Békésy 1941) schwer zu vereinbaren. Vielleicht wurde die Hydrodynamik des Endolymphschlauches durch die zur stroboskopischen Beobachtung der Reissner schen Membran aufgelagerten Silber und Aluminiumpartikel (Dichte 10,5 bzw 2 7 g/cm³) verändert.

Ein Vergleich der Dichtewerte und von färberischen und uv photometrischen Messungen mit der Ultrastruktur des membranösen Labyrinthes (Spoendlin 1957 Iurato, 1960 1967) zeigt, daß die Trockenmasse pro Volumeneinheit wesentlich von der Packung der Fibrillen abhängt. Die Tectorialmembran weist bei niedriger Dichte und Färbbarkeit der dibasischen (NGS) und aromatischen Aminosäuren (Millon und UV Extinktion) einen relativ hohen Gehalt an reagierenden Sulfat und Carboxyl gruppen auf (GC) was auf ein Überwiegen der Polysaccharid Proteinkomplexe der Grundsubstanz (vgl Schätzle u. Mitarb., 1963 Kuhn u Mitarb., 1968) schließen läßt Auch die niedrigere Dichte der BM in den oberen Windungen steht in guter Übereinstimmung mit der geringeren Anzahl der Fibrillen nach elektronenmikroskopischen Beobachtungen (Iurato, 1962) der Gehalt an Tyrosin (Millon Färbung) ist erniedrigt, die GC Konzentration erhöht.

Am Pfeilerkopf findet sich ferner bei hohen UV Extinktionen eine sehr starke Färbbarkeit mit *Millon s Reagens*. Der Befund spricht für einen hohen prozentualen Gehalt der Filamente der Pfeilerzellen an Tryptophan und besonders Tyrosin (vgl Schätzle u Mitarb., 1966) Da das membranöse Labyrinth bei 275 nm im Mittel fünf bis sechsfach höhere Extinktionen als kollagenes Sehnengewebe auf

weist, ist ein hoher Tyrosin-Tryptophangehalt nicht nur für die Fibrillen des *lig. spirale* (Iurato, 1962) und der Tectorialmembran (Iurato, 1960) sondern des gesamten membranösen Labyrinthes anzunehmen.

Die normal-logarithmische Verteilung der Dichtewerte könnte durch einen methodischen Systemfehler bedingt sein. Die Substanzkonzentration wurde nach einer linearen Proportionalität zwischen Brechungsindex und Dichte (Gladstone-Dale, 1863) errechnet, während Lorentz u. Lorenz (1880) eine exponentielle Relation annehmen. Doch wurde auch bei elektronenmikroskopischen Bestimmungen der Trockenmasse von Mitochondrien (Glas u. Mitarb., 1966) und Spermien (Bahr u. Mitarb., 1964) eine gleichartige Verteilung nachgewiesen, deren biologische Bedeutung zur Zeit unbekannt ist.

Die angedeutet exponentielle Abnahme der Substanzkonzentration während Reifung und Alterung steht im Widerspruch zur Physiko-Chemie der Alterung von Sehnen. Bei reifendem und alterndem kollagenen Stützgewebe kommt es neben einer Reduzierung der Stoffwechselintensität (Hauss u. Mitarb., 1961) und typischen Änderungen in der Molekularstruktur der Polysaccharid-Proteinkomplexe der Grundsubstanz (Buddecke 1958 Kröz u. Mitarb 1967) zu einer relativen Zunahme des Kollagenanteils (Übersicht. Clausen, 1966), indem die kristalline Fraktion ansteigt (Yan Lin u. Mitarb 1968) Bei zunehmendem Vernetzungsgrad resultiert eine verringerte Hydratation, die Dichte steigt an (Bürger 1960, 1961 Übersichten. Sobel u. Mitarb., 1957 Hall, 1968 Bailey 1968)

Wie weit die Dichteabnahme der BM als Zeichen einer „atypischen Alterung" zu deuten ist oder durch besonders hohe Substanzverluste bei der Entkalkung von reifen und senilen Innenohren zu erklären ist, die den normalen Alterungsprozeß maskieren, muß offen bleiben. Auch am Glaskörper nimmt im Alter der Wassergehalt zu bei reduziertem Sediment, Ca- und Eiweißkonzentration (Bembridge u. Mitarb 1951 Bürger u. Mitarb., 1958). Der sichtbaren

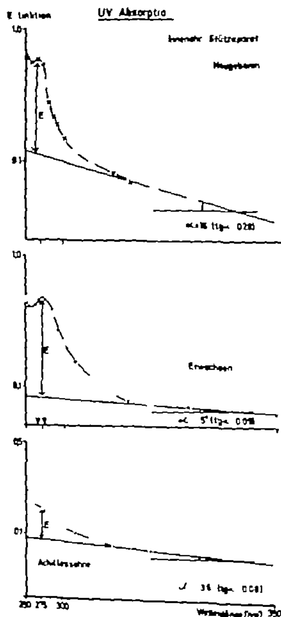


Abb 6 Graphische Darstellung der UV-Extinktionen des membranösen Labyrinthes an neugeborenen (*oben*) und reifen Meerschweinchen (*Mitte*) im Vergleich zur Achillessehne von reifen Tieren (*unten*) im doppeltlogarithmischen Raster Abszisse: Wellenlänge in nm, Ordinate: Extinktion. *E* = konsumptive Extinktion nach Streulichtkorrektur mit der Geraden der Steigung tgα.

Vakuolisierung (Goldmann, 1967 vgl. Hollwich, 1967) entspricht offenbar ein erniedrigter Brechungsindex (Steindorff 1925) Andererseits kann bei der Ratte die zitratlösliche

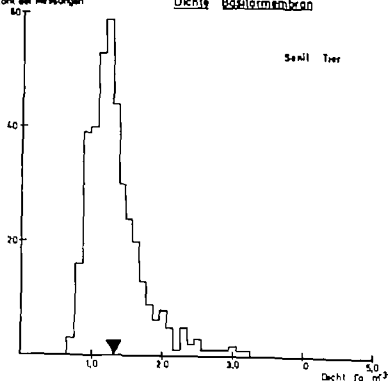


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Am Pfeilerkopf findet sich ferner bei hohen UV Extinktionen eine sehr starke Färbbarkeit mit Millon s Reagens. Der Befund spricht für einen hohen prozentualen Gehalt der Filamente der Pfeilerzellen an Tryptophan und besonders *Tyrosin* (vgl. *Schätzle u Mitarb* 1966) Da das membranöse Labyrinth bei 275 nm im Mittel fünf bis sechsfach höhere Extinktionen als kollagenes Sehnengewebe auf

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In histologischen Schnitten der Schnecke vom Meerschweinchen wurden cytophotometrisch nach Naphtholgelb S-, Millon- und Gallocyanin-Chromalaunfärbung die Relationen der cytoplasmatischen Eiweiß- und RNA-Konzentration der Zellen des Innenohres bestimmt. Die höchste Konzentration an cytoplasmatischen Organellen und Ribosomen weisen die Ganglienzellen des *ggl. spirale* (100 %) die niedrigsten die Rezeptoren (20 bis 30 %) und Stützzellen des Corti'schen Organes (10 bis 20 %) auf. Die Meßwerte stimmen mit der Ultrastruktur des Innenohres gut überein. Die Relationen des cytoplasmatischen RNA-Gehaltes weisen weder mit der Eiweißkonzentration noch mit historadiographisch ermittelten Werten der Proteinsynthese (Literatur) Übereinstimmung auf. Der Quotient aus Aminosäure Einbaurate und relativer RNA Konzentration als Maß für die Intensität der ribosomalen Eiweißsynthese ist in den Zellen des Corti schen Organes drei-bis siebenfach hoher als bei Ganglienzellen des *ggl. spirale* Striaepithelien, Fibro- und Osteozyten. Die Meßergebnisse sprechen für eine zellspezifische Intensität der ribosomalen Proteinsynthese. Als Ursache werden cytoplasmatische Faktoren wie Kationenkonzentration, Aminosäurespiegel, pH-Wert und Kontaktinhibition vermutet.

Neben einer Kumulierung von Streptomycin wird die hohe Intensität der ribosomalen Peptidsynthese im Corti'schen Organ als prädisponierend für die Ototoxizität dieses Antibiotikums angesehen. Da äußere Haarzellen gegenüber Streptomycin am empfindlichsten sind, aber die höchste ribosomale Syntheserate den Stützzellen zukommt, werden darüber hinaus spezifische Faktoren im Stoffwechsel der Haarzellen vermutet.

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Quantitative cytochemistry of the inner ear of the young and senile guinea pig

Histological sections of the cochlea of the guinea-pig were stained with naphtol yellow S Millon's reagent and gallocyanin-chrome alum, and the relations of the cytoplasmatic protein and RNA concentration of the cells in the inner ear were determined by cytophotometry The highest concentration of cytoplasmatic organellas and ribosomes is shown to be in ganglion cells of the spiral ganglion (100%), and the lowest concentration is found in the receptors (20–30%) and the supporting cells of the organ of Corti (10–20%). There is a fair degree of correspondence between the test results and the ultrastructure of the inner ear The relative cytoplasmatic RNA content shows no correlation with the protein concentration or with protein synthesis data (see lit.) obtained by autoradiography The quotient of amino acid incorporation rate and RNA concentration as a measure of ribosomal activity is between three and seven times higher in the cells of the organ of Corti than in the ganglion cells of the spiral ganglion, epithelium of the vascular stria, fibro- and osteocytes, suggesting a cell-specific intensity of ribosomal protein synthesis, due to cytoplasmatic factors such as cation concentration, the amino acid level, pH value and contact inhibition.

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The density of the basilar membrane shows a normal-logarithmic distribution. It decreases from 1.76 g/cm³ in new-born animals to 1.33 g/cm³ in mature and to 1.29 g/cm³ in senile animals. The basilar membrane thus seems to age like the vitreous body while in maturing collagen tissues there is an increase in density Assuming a water content of about 80% one can calculate density values of the basilar membrane *in vivo* of 1.1 to 1.2 g/cm³ on the basis of which a uniform wave motion of the cochlear duct is considered unlikely

Ultraviolet-microspectrography shows that the average extinction of the membraneous labyrinth is six times as high as in the Achilles tendon. A high percentage of aromatic amino acids in the fibrillary proteins is suggested by the relatively high absorption of the tectorial membrane after staining with gallocyanin-chrome alum and the pillar heads after staining

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Die Befunde beweisen tiefgreifende physikochemische Strukturwandlungen am reifenden und alternden membranösen Labyrinth. Mor phologische Anhaltspunkte für eine bisher hy pothetische Basilarmembranschwerhörigkeit werden *aufgeführt* und die Meßergebnisse als Anzeichen veränderter Hydrodynamik während Wachstum und Alterung angesehen, ohne die Existenz dieses Typs von Innenohrschwer hörigkeit beweisen zu können.

Summary

Quantitative cytochemistry of the inner ear of the young and senile guinea pig

Histological sections of the cochlea of the guinea-pig were stained with naphtol yellow S, Millon's reagent and gallocyanin-chrome alum, and the relations of the cytoplasmatic protein and RNA concentration of the cells in the inner ear were determined by cytophotometry The highest concentration of cytoplasmatic organelles and ribosomes is shown to be in ganglion cells of the spiral ganglion (100%), and the lowest concentration is found in the receptors (20–30%) and the supporting cells of the organ of Corti (10–20%). There is a fair degree of correspondence between the test results and the ultrastructure of the inner ear The relative cytoplasmatic RNA content shows no correlation with the protein concentration or with protein synthesis data (see lit.) obtained by historadiography The quotient of amino acid incorporation rate and RNA concentration as a measure of ribosomal activity is between three and seven times higher in the cells of the organ of Corti than in the ganglion cells of the spiral ganglion, epithelium of the vascular stria, fibro- and osteocytes, suggesting a cell-specific intensity of ribosomal protein synthesis, due to cytoplasmatic factors such as cation concentration, the amino acid level, pH-value and contact inhibition.

In addition to an accumulation of streptomycin, the high intensity of ribosomal peptid synthesis in the organ of Corti is considered to be a predisposing factor for the ototoxicity of this antibiotic. Since outer hair cells are most sensitive to streptomycin, but the supporting cells have the highest rate of synthesis, further specific factors in the metabolism of the hair cells are suspected.

According to interferometric measurements with the two-medium method the pillar-heads show the highest density in histological sections (1.41 g/cm³) then follow the spiral ligament (1.35 g/cm³) the basilar (1.33 g/cm³) and the tectorial membrane (1.20 g/cm³). The basilar membrane is more compact in the lower turns than in the upper turns. The test results are within the same order of magnitude as the density of chemical constituents of connective tissues and organelles of the cell. A comparison with the ultrastructure of the membraneous labyrinth shows that the density values depend on the concentration of fibrils.

The density of the basilar membrane shows a normal-logarithmic distribution. It decreases from 1.76 g/cm³ in new-born animals to 1.33 g/cm³ in mature and to 1.29 g/cm³ in senile animals. The basilar membrane thus seems to age like the vitreous body while in maturing collagen tissues there is an increase in density Assuming a water content of about 80% one can calculate density values of the basilar membrane *in vivo* of 1.1 to 1.2 g/cm³ on the basis of which a uniform wave motion of the cochlear duct is considered unlikely

Ultraviolet-microspectrography shows that the average extinction of the membraneous labyrinth is six times as high as in the Achilles tendon. A high percentage of aromatic amino acids in the fibrillary proteins is suggested by the relatively high absorption of the tectorial membrane after staining with gallocyanin-chrome alum and the pillar heads after staining

with Millon's reagent with strong UV absorptions

During maturing the average absorption maximum of the membraneous labyrinth shifts from 270 to 277 nm and the gradient of the correction-line for light scattering drops from 24 to 19 This may be caused by differences in the size of molecules, their dissociation and changed chemical composition of the tissue

The findings prove profound physico-chemical structural changes in the maturing and ageing membraneous labyrinth. They indicate hydrodynamic changes during growth and ageing but cannot prove the existence of a basilar membrane deafness.

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BY

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CONTENTS

Introduction

Acetylcholinesterase (AChE) activity was demonstrated in the inner ear for the first time in the perilymph of the pigeon by MARTINI (1941) with the biological method of CHANG and GADDUM (1933), then biologically and by acidity tests by GISSELSSON (1950) in the perilymph and endolymph of the cod pigeon cat and guinea pig.

Later AChE activity was histochemically localized in the cochlea by many authors with the light microscope both in normal and experimental conditions (see Tables 1 and 2). A more detailed localization of AChE in the cochlea was obtained by the electron microscope (see Table 3)

AChE activity was also histochemically demonstrated in the vestibular sensory areas and vestibular nerve with the light and electron microscopes (see Table 4).

It was the aim of the present research to obtain a better understanding of the localization of AChE activity in the inner ear. Preliminary results were reported at the Symposium on Biochemical Mechanisms in Hearing and Deafness held in Minnesota in August 1968 (IURATO et al. 1970) and at the Collegium O.R.L.A.S meeting held in Palermo in September 1970 (IURATO et al. 1971 in press).

Introduction

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contd Table 1

Authors	Year	Method	Material	Results
Ishii & Balogh	1968	Gomori	cat	P. R. at the outer hair cells decreased towards apex, particularly in outer row. Near apical end all three rows of outer hair cells lacked P. R. Another area of low P. R. at extreme basal end of organ of Corti.
Firbas & Welleschik	1970	Karnovsky	bat	P. R. on efferent nerve fibers and endings and on cochlear ganglion cells. Scarce P. R. in first half of basal turn and in apical turn.

Table 2. *Histochemical AChE localization in the cochlea using light microscopy*
(Experimental animals)

Authors	Year	Method	Material	Experiment	Result
Churchill & Schuknecht; Schuknecht et al.	1959; 1959	Koelle	cat	destruction of hair cell by streptomycin	Positive reaction (P. R.) not appreciably decreased in organ of Corti.
				section of olivo-cochlear bundle (OCB)	P. R. markedly decreased. Presence of AChE activity was first related to efferent OCB fibers.
Conti	1961	Koelle	guinea pig	acoustic trauma	P. R. markedly reduced or negative reaction (N. R.) in organ of Corti, only in turns acoustically stimulated.
Vinnikov & Titov	1958; 1961; 1962; 1963; 1964	Koelle, Friedenwald modified by Gerebtzoff	cat	acoustic trauma	P. R. increased after 30 min exposure, but markedly decreased after longer exposures, in organ of Corti in turns acoustically stimulated.
Rossi & Cortesina	1966	Koelle Friedenwald	guinea pig	destruction of organ of Corti and cochlear ganglion by acoustic trauma + neomycin sulphate	N. R. in cochlear duct. P. R. in intraganglionic spiral bundle.
Mounier-Kuhn & Haguenau	1967	Koelle Friedenwald	guinea pig	acoustic trauma	P. R. decreased in organ of Corti (1st and 2nd turn) after prolonged exposure to high frequency sounds.

Table 1 *Histochemical AChE localization in the cochlea using light microscopy*
(Normal animals)

Authors	Year	Method	Material	Results
CHURCHILL et al. CHURCHILL & SCHUKNECHT SCHUKNECHT et al	1956 1959 1959	KOELLE	cat	Positive reaction (P R) in organ of CORTI on nerve fibers and at base of hair cells. Densest stain in the upper basal turn, progressively decreasing toward basal and apical ends. Negative reaction (N R) in cochlear ganglion cells.
ROSSI	1961	KOELLE FRIEDENWALD	guinea pig	During development P R in organ of Corti and in intraganglionic spiral bundle first appears in basal turn and later extends to others. N R in cochlear ganglion cells except for some early stages of development.
DEL BO & CONTI	1961	KOELLE FRIEDENWALD	guinea pig	P R in cytoplasm of hair cells near nerve endings and along non-myelinated nerve fibers in organ of Corti. During development P R first appears in basal turn
ROSSI & CORTESINA	196.	KOELLE FRIEDENWALD	guinea pig	P R on cochlear efferent fibers in brain stem
VINNIKOV & TITOVA	1958 1961 196. 1963	KOELLE FRIEDENWALD modified by GEREBTZOFF	cat	Intense P R on basal surface of hair cells at sites of their contact with fibers and endings of inner and outer spiral bundles. Marked P R in hairs of inner hair cells throughout the whole length of organ of Corti and in hairs of outer hair cells at the level of basal turn and of lower part of middle turn. Low P R in cytoplasm of hair cells and cochlear ganglion cells.
VINNIKOV et al	1965	KOELLE FRIEDENWALD modified by PEARSE or GEREBTZOFF or HOLMSTEDT	reptilians, birds mammals	Intense P R in nerve endings, in cytoplasm and hairs of hair cells.
NOMURA & SCHUKNECHT	1965	GOMORI	cat, man	P R in intraganglionic spiral bundle and in osseous spiral lamina related to efferent fibers
GACEK et al	1965	GOMORI	cat	P R on efferent cochlear fibers traveling entirely within cochlear nerve toward upper middle and apical turns.
ISHII et al	1967 a	GOMORI	man, monkey cat guinea pig	P R on nerve fibers, not belonging to OCB scattered within cochlear nerve trunk. P R in efferent nerve fibers in osseous spiral lamina on tunnel radial fibers and outer spiral bundles. P R in cochlear ganglion cell of man but not of monkey cat and guinea pig
ISHII et al.	1967 b	GOMORI	squirrel monkey	P R at the inner spiral bundle, 3 outer spiral bundles, upper and lower tunnel radial fibers. N R in cochlear ganglion cells.

Table 4 *Histochemical AChE localization in the vestibular sensory areas*

Authors	Year	Method	Material	Results
DOHLM[illegible] et al.	1958	KOELLE FRIEDENWALD modified by HOLMSTEDT	pigeon	Positive reaction (P R.) at base of the neuroepithelium of cristae ampullares and macula utriculi and at nerve endings between hair cells. Presence of efferent cholinergic fibers.
DOHLMAN	1960	KOELLE FRIEDENWALD modified by HOLMSTEDT	pigeon	High P R. in vestibular sensory areas below neuroepithelium. High butyrylcholinesterase activity in same areas. Could it possibly suggest that butyrylcholine was the chemical mediator for afferent receptor system and acetylcholine belongs to efferent endings
IRELAND & FARKASHIDY	1961	KOELLE FRIEDENWALD	guinea pig	P R. localized at nerve endings and fibers below and between hair cells in ampullae of all three semicircular canals and macula utriculi. Negative reaction (N. R.) when auditory nerve [illegible] sectioned.
ROSSI	1961	KOELLE FRIEDENWALD	guinea pig	N. R. in macula utriculi, macula sacculi and cristae ampullares of semicircular canals during fetal development and in young animals. During development unspecific ChE activity was present; it disappeared before birth at level of maculae utriculi and sacculi and in adult animals at level of cristae ampullares. Constant P R. in cells of vestibular ganglion during development and in adult animals. P R. in nerve fiber bundles in central branch of vestibular nerve.
HILDING & WERSÄLL	196[illegible]	KOELLE FRIEDENWALD modified by HOLMSTEDT applied to EM	guinea pig	Using electron microscope black precipitate was found in the small granulated nerve endings at the bottom of type II hair cell and in nerve endings on outside of nerve chalices of type I cell. Using light microscope after incubation with BW C 51 for demonstrating butyrylcholinesterase diffuse staining of cristae ampullares more intense in planum semilunatum.
ROSSI & CORTESINA	196[illegible]	KOELLE FRIEDENWALD	guinea pig	P R. in fiber bundles in branches of vestibular nerve.
ROSSI et [illegible]	1964	KOELLE FRIEDENWALD	rabbit	N R. throughout efferent vestibular fiber system and at cristae ampullares after arterial injection of diisopropylfluorophosphate (DFP). P R. after restoration in normal conditions.

Table 3 Histochemical AChE localization in the cochlea using electron microscopy

Authors	Year	Method	Material	Results
Wersäll et al Hilding & Wersäll	1961 1962	Koelle Friedenwald modified by Holmstedt applied to EM	guinea pig	Positive reaction (P R.) in inner spiral bundle and under outer hair cells of all rows in basal turn, of first and second row in middle turn and only of first row in apical turn Same localization as that of efferent nerve endings.
Uchizono et al	1967	Karnovsky	man (2 hr post-mortem)	P R on axolemma of myelinated and non-myelinated cochlear nerve fibers in osseous spiral lamina
Kaneko Kaneko & Daly	1968 1968	Karnovsky	guinea pig	P R on upper tunnel radial fibers and efferent nerve endings to outer hair cells, Negative reaction (N R) on lower tunnel radial fibers.
Kaneko & Daly	1969	Karnovsky	guinea pig	P R. on endolymphatic surface of outer hair cells. N R. on surface of supporting cells.

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KANEKO KANEKO & DALY	1968 1968	KARNOVSKY	guinea pig	P R on upper tunnel radial fibers and efferent nerve endings to outer hair cells. Negative reaction (N R) on lower tunnel radial fibers.
KANEKO & DALY	1969	KARNOVSKY	guinea pig	P R on endolymphatic surface of outer hair cells. N R on surface of supporting cells.

Materials and Methods

Two adult guinea pigs and five adult chinchillas of both sexes were anesthetized with an endoperitoneal injection of sodium pentobarbital (35mg/kg). The inner ear was prefixed *in vivo* by a 10 min perilymphatic perfusion of the fixative at room temperature (IURATO and DE PETRIS, 1967). The animal was sacrificed and immediately after the temporal bones were quickly removed. The bony cochlea was completely opened and the stapes taken away to allow a good penetration of the fixative. The specimens were left for 3 hr at 0°–4°C in the same fixative used for the perfusion.

Fixation was done in 4 formaldehyde obtained from paraformaldehyde (Fluka A. G Bucks, St Gallen) according to KARNOVSKY (1965) or in 3 glutaraldehyde (Biological Grade, Fisher Scientific Co., Fair Lawn, N J) The aldehydes were diluted in the following buffers

0 075 M phosphate buffer prepared according to SORENSEN the buffer contained 0 44 M sucrose

isotonic phosphate buffer prepared according to MILLONIG (1961) the buffer contained one drop of a 1 $CaCl_2$ solution every 10 ml

0 08 0 1 M sodium cacodylate buffer containing one drop of a 1 CaCl solution every 10 ml

During fixation the bony covering of the cochlea was partially removed and the vestibulum opened the cristae ampullares and maculae utriculi and sacculi were identified and partly dissected

After fixation was completed the specimens were placed in the same previously used buffer or in a 0 44 M sucrose for 1 12 hr and the dissection of the cochlea and vestibular sensory areas was continued. Part of the specimens were then transferred into the incubation medium (substrate acetylthiocholine iodide, Hoffmann La Roche & Co Ltd., Basel) prepared according to KARNOVSKY (1964) and kept on ice For specimens incubated *in toto* the period of incubation varied from 20-40 min,

On part of the material the following controls were made

a) Incubation for 40 min in the medium lacking substrate.

b) Pre-incubation at 0°-4°C for 1 hr in 0 44 M sucrose pH 6 containing a specific inhibitor As inhibitors we used 1×10^{-4} M eserine (eserine sulfate, Merck A. G., Darmstadt) or $3\times10^{-}$ M DFP (di-isopropylphosphorofluoridate B.D H Laboratory Chemicals Division, Poole) or $1\times10^{-3}-2\times10^{-4}$ M BW 284 C 51 ([1 5-bis (4-allyldimethylammoniumphenyl) pentan-3-one dibromide] Wellcome Research,

Chinchillas used in Italy were kindly supplied by Italian Society of Chinchilla, Florence. Chinchillas used in Germany were kindly supplied by Chinchilla-Zucht und Versuchsfarm J Wolf Onolzheim.

contd Table 4

Authors	Year	Method	Material	Results
Rossi et al	1964 *b*	Koelle Friedenwald	rabbit	After local injection of DFP N R in injected cristae ampullares. P R after restoration in normal conditions
Gacek et al	1965	Gomori	cat	Efferent vestibular fibers were more numerous than previously thought *They branch as they course peripherally* in the individual vestibular nerve rami After vestibular nerve section N R in every branch of vestibular nerve
Nomura et al	1965	Gomori	guinea pig	P R at base of sensory epithelium on slopes of crista ampullaris rather than on crest P R in ampullar nerves in axons of fine caliber fibers scattered throughout nerve bundle P R. in neuroepithelium of maculae sacculi and utriculi. Less activity in striola located in middle part of macula sacculi P R. in fibers scattered throughout nerves to saccule and utricle
Ishii et al	1967 *b*	Gomori	squirrel monkey	High P R in vestibular efferent bundles N R in Scarpa's ganglion cells. P R in fine efferent fibers distributed throughout peripheral vestibular organs.

Materials and Methods

Two adult guinea pigs and five adult chinchillas of both sexes were anesthetized with an endoperitoneal injection of sodium pentobarbital (35mg/kg). The inner ear was prefixed *in vivo* by a 10 min perilymphatic perfusion of the fixative at room temperature (IURATO and DE PETRIS, 1967). The animal was sacrificed and immediately after the temporal bones were quickly removed. The bony cochlea was completely opened and the stapes taken away to allow a good penetration of the fixative. The specimens were left for 3 hr at 0°–4°C in the same fixative used for the perfusion.

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b) Pre-incubation at 0°-4°C for 1 hr in 0 44 M sucrose pH 6 containing a specific inhibitor. As inhibitors we used 1×10^{-4} M eserine (eserine sulfate Merck A. G Darmstadt) or 3×10 M DFP (di-isopropylphosphorofluoridate B D H Laboratory Chemicals Division, Poole) or 1×10^{-5} – $?\times10^{-4}$ M BW 284 C 51 ([1 5-bis (4-allyldimethylammoniumphenyl) pentan-3-one dibromide] Wellcome Research

Chinchillas used in Italy were kindly supplied by Italian Society of Chinchilla, Florence. Chinchillas used in Germany were kindly supplied by Chinchilla-Zucht and Versuchsfarm I. Wolf, Onolzheim.

Laboratories, Beckenham) or 1×10^{-5} – 2×10^{-4} M iso-OMPA (tetraisopropylpyrophosphorami de Koch Light Laboratories Ltd. Colnbrook). The incubation of this material was performed in a complete medium to which the inhibitors were added at the same concentration as used for the pre incubation. At these concentrations eserine completely inhibits AChE and ChE. DFP and iso-OMPA selectively inhibit the unspecific ChE. BW 284 inhibits AChE (BAYLISS and TODRICK 1956, PEARSE 1961, KOELLE and GROMADZKI 1966).

c) Pre incubation of the material in 1×10^{-4} eserine followed by incubation in a complete medium containing the inhibitor and having been prepared for at least 5 hr.

d) Incubation in a complete medium for 40 min of frozen and thawed specimens.

e) From other specimens 120 μ thick sections were obtained using a freezing microtome. The sections were put into KARNOVSKY's incubation medium for 10 min.

Following incubation the specimens were rinsed in 4 changes of cold 0.44 M sucrose for 10 min. They were then postfixed in a 1% osmium tetroxide buffered according to MILLONIG (1961) or with s-collidine (Eastman Organic Chemicals, Rochester, N.Y.). Dehydration was carried out in alcohol and embedding in Epon 812.

Thin sections cut with ultramicrotomes Sorvall MT I or LKB 4800 III were collected on Formvar-carbon membranes (DOWELL 1964, REALE and LUCIANO 1965) and stained by REYNOLDS (1963) or VENABLE and COGGESHALL's (1965) methods. The thin sections were examined in Siemens Elmiskop I and IA and Zeiss EM 9A electron microscopes. One μ thick sections obtained from the material embedded in Epon and mounted in glycerin were observed and photographed by phase contrast (Leitz Ortoplan).

Results

a) Preservation of the structure

The preservation of the organ of CORTI was constantly satisfactory after fixation both in glutaraldehyde and formaldehyde. The best results were obtained in material fixed in glutaraldehyde and incubated *in toto*.

On the contrary the vestibular sensory areas were not always well preserved. A remarkable shrinkage was often observed in the cytoplasm of the sensory cells whereas the nerve chalices were swollen. The best results were obtained in material fixed in formaldehyde and incubated *in toto*.

The preservation of the tissues was very poor in the frozen and thawed materials and in those sectioned using the freezing microtome. Breakdown of membranes, cytoplasmic retraction, vacuoles and mitochondrial alterations were frequently observed in all the tissues which were badly disrupted. Greater alterations were noted in the sensory cells type I and in the nerve chalices which were quite often expanded.

b) Histochemical localization of the reaction product in the organ of CORTI

Under the phase contrast microscope the organ of CORTI appeared to be the only portion

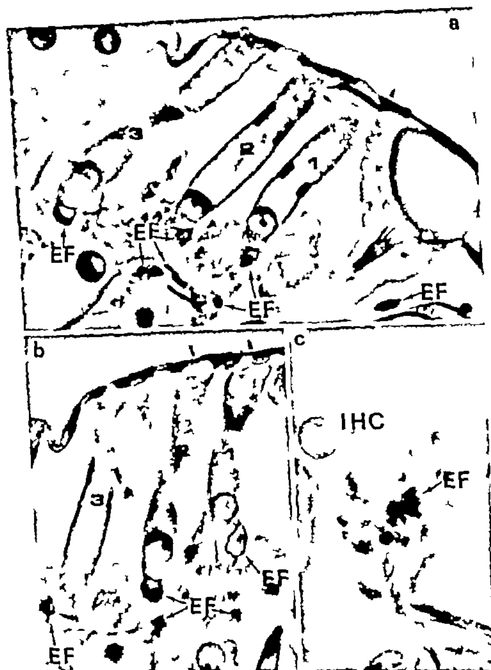


Fig. 1. Organ of Corti of chinchilla examined by phase contr 1 nd *b* the reaction product is localized below the outer hair cells (1, 2, 3) on the contour of the large efferent nerve endings (900 ×). *EF* = efferent fibers or endings. In dense reaction product can be seen below an inner hai cell (IHC). 1400 × Glutaraldehyde fixed material.

of the cochlear duct showing enzyme activity The reaction end product (Cu ferrocyanide) was clearly recognizable at the level of the inner spiral bundle below the inner hair cells (Fig. 1 c) the upper radial tunnel fibers (Fig 1 a) and some nerve endings below the outer hair cells (Fig. 1 a and b)

With the electron microscope the pictur was clearer and more precise The reactio product, which under the light microscope wa evident below the inner hair cells (Fig. 1 c) appeared localized in the inner spiral bundl (Fig. 2, 3 4 5) on the axolemma of the smal efferent fibers and of their presynaptic enlarge



F g The dense rea t m pr duct found nder the nner hair cell (IHC) is localized on the uter surf e f the small efferent f bers belonging t the nner pir l bundle (ISB) SC = pport g ell IP = m ner pillar ell E = endolymphatic pace 6,760 × Chinch lla glutaraldehyde fi ed material



Fig. 3. The small efferent fibers (EF) belonging to the inner spiral bundle and their enlargement (EL) show positive reaction on their plasma membrane. The large afferent radial fibers (AF) innervating an inner hair cell (IHC) appear negative except where they contact the efferent enlargements. The gap between the ending of one of these fibers and the plasma membrane of the inner hair cell is devoid of the reaction product (arrow). 24 000 ×. Chinchilla glutaraldehyde fixed material.

of the cochlear duct showing enzyme activity The reaction end product (Cu ferrocyanide) was clearly recognizable at the level of the inner spiral bundle below the inner hair cells (Fig 1 *c*) the upper radial tunnel fibers (Fig 1 *a*) and some nerve endings below the outer hair cells (Fig. 1 *a* and *b*)

With the electron microscope the picture was clearer and more precise The reaction product which under the light microscope was evident below the inner hair cells (Fig. 1 *c*), appeared localized in the inner spiral bundle (Fig. 2 3 4 5) on the axolemma of the small efferent fibers and of their presynaptic enlarge



Fig. 2 The dense react product fou d nder th nner hair cell (IHC) is localized on the ut r urf ce of the small efferent f bers belonging to the nner piral bundle (ISB). SC = supporting cell IP n ner pillar cell E = endolymph tic pace 6,760 × Ch n h lic glutar ldehyde f xed material



Fig. 4. The picture shows at higher magnification (60,000×) an afferent radial fiber (AF) in contact with several small efferent fibers (EF) and enlargement (EL). Shape and position of dark granules of the reaction product are visible. Subsequent sections of the contoured areas are shown in Fig. 5 and 6. Chinchilla, glutaraldehyde fixed material.

ments (Fig. 3 4) The reaction product was absent at the large afferent radial fibers (den drites of the cochlear ganglion cells) innerva ting the inner hair cells, except where they contact the efferent enlargements (Fig. 3 4 5) The endings of these afferent fibers were also devoid of the reaction product as well as the gap between them and the plasma membrane of the inner hair cells (Fig. 3)

The upper tunnel radial fibers (Fig. 6 *a* and *b*) and those of the spiral tunnel bundle showed a heavy precipitate on their outer surfaces. The reaction product was absent on the lower tunnel radial fibers (Fig. 6 *a*)

The large efferent nerve endings in contact with the base of the outer hair cells (axosomatic synapses) exhibited a large amount of the reaction product (Fig 7 8) This appeared deposited on the plasma membrane of the efferent endings and filled the synaptic gap between these endings and the plasma mem brane of the outer hair cells (Fig. 8 9 *a* 9 *b*) This gap which usually measures 215 225 A, (C A SMITH 1967 *a*) often appeared irregu larly enlarged the expanded zones always being filled with granules (Fig. 9 *b*) The subsynaptic cistern located inside the outer hair cells close to the efferent terminals, did not show the *reaction product (Fig. 9a 9 b) Granules were* present in the synaptic cleft of the axodendritic synapses described by C A SMITH and RASMUSSEN (1963) and by C A. SMITH (1967 *b*) below the outer hair cells (Fig 10 11) and on the plasma membrane of the efferent fibers located in the same region (Fig. 12)

It may be of some interest that, with the histochemical methods used in the present research the synaptic vesicles contained in the efferent nerve endings always appeared oblongated Instead after osmium tetroxide fixation or glutaraldehyde fixation not followed by incubation for AChE, the same vesicles appeared spherical

Most of the outer spiral fibers (dendrites of the cochlear ganglion cells) did not show the reaction product (Fig. 12) The afferent nerve endings in contact with the outer hair cells were also without reaction end product (Fig. 10) The gap between them and the plasma membrane of the outer hair cells did not generally contain the reaction product (Fig 10) Sometimes, only a few granules could be seen in this gap (Fig. 8). This happened when one or more efferents were in contact with the afferent nerve ending (Fig. 8) and never when several nerve endings all afferent, were in contact with each other (Fig. 10).

The non myelinated fibers in the spiral lamina showed a large amount of the reaction product on their outer surface Not having done specific research in this area we could not affirm if all these fibers were positive.

In all the above mentioned areas the reaction end product (Cu ferrocyanide) was granular and the size of the single granule was variable but generally rather large

A careful examination of the micrographs revealed an amorphous or very finely granulated precipitate coating the plasma membrane of the hairs and cuticle of the inner and outer hair cells (Fig. 13) and the free surface of the body of outer hair cells and pillars (Fig. 14). In these areas the amount of the precipitate was scarce if compared with that found on the plasma membrane of the efferent fibers and endings. The plasma membrane of the myelinated fibers in the osseous spiral lamina did not show a true precipitate but only an increased electron density (Fig. 15)

c) Histochemical localization of the reaction product in the vestibular sensory areas

In the vestibular labyrinth under the phase contrast microscope the reaction product (Cu-ferrocyanide) was only localized in the sensory areas Since the localization was similar in the cristae ampullares and in the maculae utriculi and sacculi to avoid repetition a unique description is given Inside the neuroepithelium



Fig 4 The picture show at higher magnification 60,000) an afferent radial fiber (AF) in contact with several small efferent fibers (EF) and enlargement (EL) Shape and position of dark granules of the reaction product are visible. Subsequent sections of the two contoured areas are shown in Fig 5 and 6 Chinchilla, glutaraldehyde fixed material.

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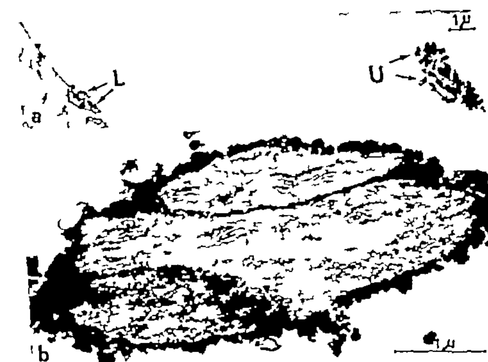
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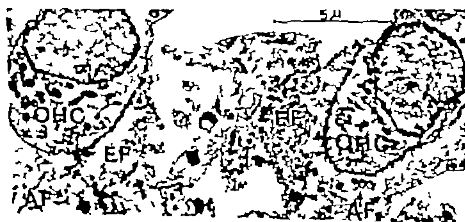
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In the vestibular labyrinth under the phase contrast microscope the reaction product (Cu-ferrocyanide) was only localized in the sensory areas. Since the localization was similar in the cristae ampullares and in the maculae utriculi and sacculi to avoid repetition a unique description is given Inside the neuroepithelium



6 In the upper tunnel radial fibers (U) show hea y precipitate on their outer surface, herea e lower tunnel radial fibers (L) are negative 190 Chinchilla glutaraldehyde fixed material I b higher magnification of the upper tunnel radial fibers can be seen 4 000 x Guinea pig. formaldehyde fixed material



Base of the outer hair cell OHC) The large efferent nerve endings EP) exhibit enzyme activity here the afferent endings AF) re negative 6 40 x Chinchilla glutaraldehyde fixed material



Fig 5 In *a* and *b* bsequent sections f the tw contoured area of f g 4 re shown The compa rison show that the gr n les of the re ction product can be differently located n the subsequent sect on AF = fferent f be I I efferent f bers El f f re t enlargement I cros sect on of large r dial ff rent f ber (AF) m contact w th se er l efferent (EF) 60 000 × Ch nch lla gluta ldehyde f ed ma t rial

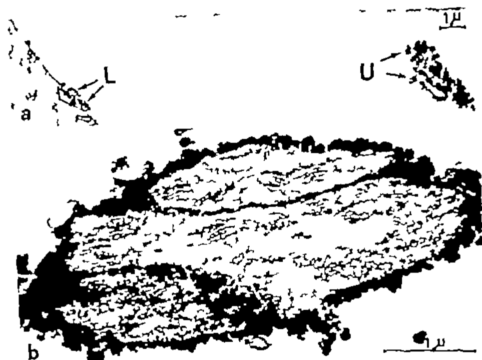


Fig. 6. In a the upper tunnel radial fibers (U) show heavy precipitate on their outer surface, whereas the lower tunnel radial fibers (L) are negative. 6,790 ×. Chinchilla, glutaraldehyde fixed material. In b higher magnification of the upper tunnel radial fibers can be seen. 4,000 ×. Guinea pig; formaldehyde fixed material.



Fig. 7. Base of two outer hair cells (OHC). The large efferent nerve endings (EF) exhibit enzyme activity, whereas the afferent endings (AF) are negative. 6,?40 ×. Chinchilla, glutaraldehyde fixed material.



F q 5 I a a d b ubsequent sect n f the tw contoured area f Fig 4 re shown The ompa rison show that the gr les of the rea tion product can be d fferently located the subsequent sect on AF = afferent f ber EF = efferent f bers EL f ferent enlargement In ross-section of large radial fferent f be (AF) ontact with sever l efferent (EF) 60,000 × Ch nch lla glutaraldehyde fixed ma t rial

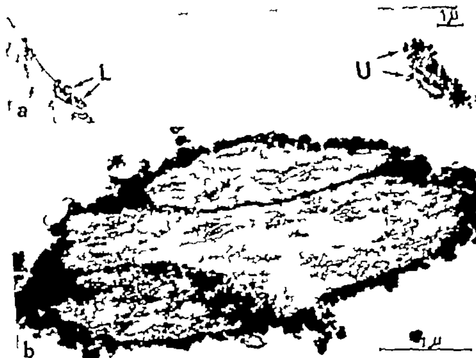


Fig 6 In the upper tunnel radial f bers (U) show he vy precipitate on thei outer surface, wherea the lower tunnel adial f bers (L) re negative 4 90 x Chinchill glutaraldehyde fixed material I b higher magnification of the upper tunnel adial fibers ca be seen 24,000 × Guinea pig formaldehyde fixed material



7 Base of t outer hair cell (OHC) The l ge efferent ner endings (E) exhibit enzyme activity here The a ent endings AF) re ne gat 6,40 Chinchilla glutaraldehyde fixed material

the reaction product was observed at the level of some nerve fibers and endings (Fig 16 22) below the basement membrane at the level of some non myelinated fibers (Fig. 16 *b* 22 *b*)

The picture was clearer under the electron microscope

The reaction product was observed on the plasma membrane of the efferent nerve endings and dilated synaptic areas along the presynaptic efferent fibers in contact with the outer surface of the nerve chalices (Fig. 17 18, 24 25) The reaction product filled the synaptic gap between these efferent synapses and the



Fig 8 Base of uter ha r cell (OHC) ntact with two ffere t (EF) nd sever l ffe ent (AF) nerve end ngs The reaction product ppe rs depo sited on the plasma membr ne f the fferent end gs nd partially f ll the synaptic gap between these endings nd the outer hair cell 19,200 × Chm ch lla gl taraldehyde f ed materi l

plasma membrane of the outer surface of the afferent nerve chalices (Fig. 18 inset, 24 b). Instead the reaction product was constantly absent in the gap between the plasma membrane of the hair cells type I and the afferent nerve chalices (Fig. 18 19 24 b 25).



Fig. 19 a and b. Small part of an outer hair cell (OHC) in contact with an efferent nerve ending (EF) are visible. The reaction product fills the synaptic gap (G) between the efferent ending and the plasma membrane of the outer hair cell. This gap appears irregularly enlarged owing to the reaction product granules. Notice that the synaptic vesicles are oblongated and that the subsynaptic cistern (SS) does not show enzyme activity. A = active point of the synapse. a 80,000 ×, b 70,000 ×. Chinchilla formaldehyde fixed material.

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F g *8* Base f n t r ha r ell (OHC) in ntact w th tw efferent (EF) nd sever l fferent (AF) nerve end ngs The re t m product appe rs deposited on the pla ma memb ne f the efferent end gs nd partially f ll the synaptic gap between these end ngs and the outer ha r cell. 19,200 × Ch ch ll gl tar ldehyde fi ed m terial

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[illegible] and *b* small parts of an outer hair cell (OHC) in contact with efferent nerve ending [illegible] are visible. The reaction product fill the synaptic gap (G) between the efferent ending and the plasma membrane of the outer hair cell. This gap [illegible] irregularly enlarged owing to the reaction product granules. Notice that the synaptic vesicles are oblongated and that the subsynaptic cistern (SS) does not show enzyme activity. A = active points of the synapse. *a* 80,000 ×, *b* 70,000 ×. Chinchilla, formaldehyde fixed material.

The enzymatic activity was observed on the plasma membrane of the efferent nerve endings at the base of hair cells type II (Fig 20 24 *a*) The afferent nerve endings did not generally show the reaction product In fact the gap between them and the plasma membrane of the hair cells type II was generally free of precipitate (Fig 24 *a*) Sometimes only a few granules could be seen in this gap (Fig. 20) The subsynaptic cistern, located inside the hair cells type II close to efferent terminals did not show enzymatic activity (Fig. 20 inset) In some cases the efferent nerve endings showing the reaction product were not in direct contact with the base of the hair cells but with one or more afferent nerve endings (Fig 24 *a*)



Fig 10 Base of an outer hair cell (OHC) in contact with several afferent nerve endings (AF) all negative Below a positive efferent nerve ending (EF) visible 4000 × Chinchilla glutaraldehyde fixed material



Fig. 11 1 base of outer hair cell (OHC) in contact with fferent and efferent (EF) nerve endings. An xodendritic synapse is boxed. The boxed rea in may be seen at higher magnification in *b*. EF = efferent ending. AF = afferent fibers. The boxed area in *b* is f rther enlarged in to show active point (A) and the reaction product n the synaptic interval 7,800 × *b* 18,400 × 60,000 × Chinchilla gluta aldehyde fixed material.

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Fig. 10. Base of an outer hair cell (OHC) in contact with several afferent nerve endings (AF) ... negative. Below ... positive efferent nerve ending (EF) ... visible. 4000 ×. Ch... glutaraldehyde fixed material.



F 11 In base of an outer hair cell (OHC) in contact ith afferent and efferent (EF) nerve endings An axodendritic synapse boxed The boxed area in may be seen t higher magnification in *b* EF = efferent ending AF = afferent fibers The boxed area in *b* is *further enlarged in* to show active points (A) and the reaction product in the synaptic interval 7,800 × *b* 18,400 × 60,000 × Chinchilla glutaraldehyde fixed material

The intraepithelial efferent fibers constantly showed the reaction product on their outer surface (Fig 19 21 *a* 25 *b*) In places where these fibers enlarge they make axodendritic synaptic contacts with the afferent nerve fibers (vestibular nerve dendrites) Reaction product was always evident in the cleft of these axodendritic synapses (Fig 19 ellipse 23 inset).

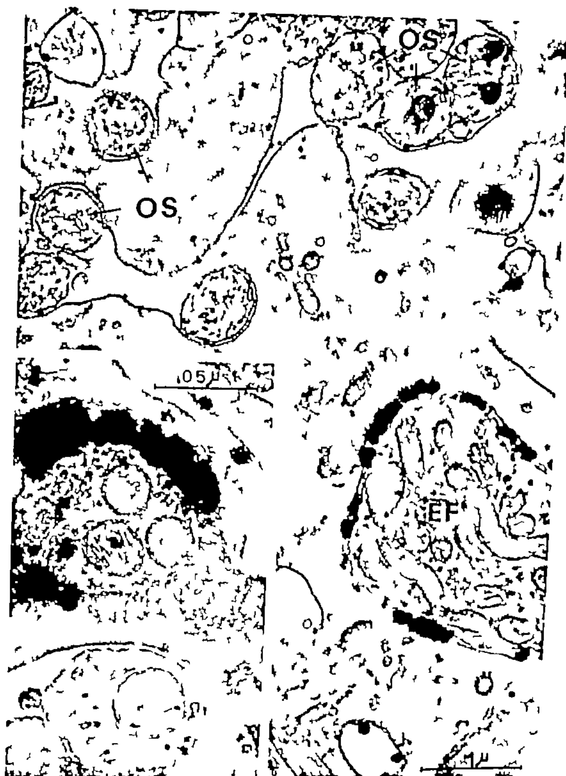


Fig 12 Several outer spiral fibers (OS), which are afferent, appear negative. An efferent positive fiber (EF) is also visible (4,000 ×). The inset (46,000 ×) shows two fibers, one positive and one negative. Chinchilla glutaraldehyde fixed material



Fig. 13 In (incubation in complete medium) very fine precipitate can be seen on the plasma membrane of the hairs (H) and the cuticle (C) of an outer hair cell (arrows). The plasma membrane of the supporting cells (S) negative. In controls *b* (incubation without substrate), (incubation in presence of BW 284 and *d* (incubation in presence of iso-OMPA) the same results were obtained. Chinchilla formaldehyde fixed material. *a*, *d* 24,000 × *b* 60,000 ×

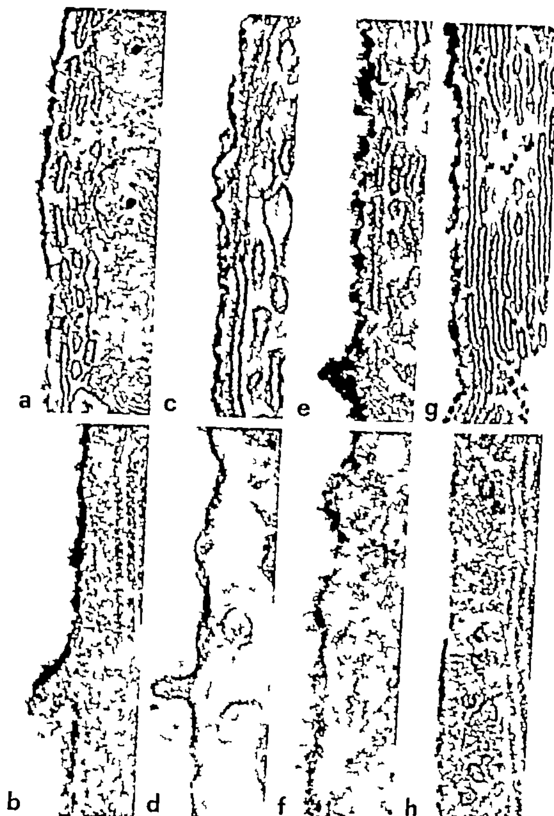


Fig. 14. Free surface of t body f uter hai cells (c) nd p llars f h). In *a* nd *h* (incubatu mpl um), d *d* (incubation without bst ( ) d *f* (ncubation n presence of BW 284), *g* nd *h* (incubation in presence iso-OMPA) the same result were obtained. Chi chilla f rmaldehyde fixed material (60,000 ×).

Below the basement membrane the reaction product was localized on the non-myelinated fibers (Fig. 21 *b* 26). In the examined areas all these fibers were positive

In all the above mentioned areas the reaction product was abundant and the size of the single granules rather large

Under the same experimental conditions a scarce amorphous or very finely granulated precipitate was observed along the plasma membrane of the hairs and cuticle of the sensory cells (Fig. 13).

d) Control studies

Different results were obtained in the areas where the precipitate was (α) granular and (β) amorphous or very finely granulated

(α) The material incubated without substrate (control *a*) did not show any specific precipitate. The material incubated in a stale medium (control c) showed many unspecific precipitates spread all over the material No reaction was obtained in the control *b* i e in the presence of 1×10^{-4} eserine AChE competitive inhibitor or $1 \times 10^{-} - 2 \times 10^{-4}$ M BW 284 C 51 AChE irreversible inhibitor in

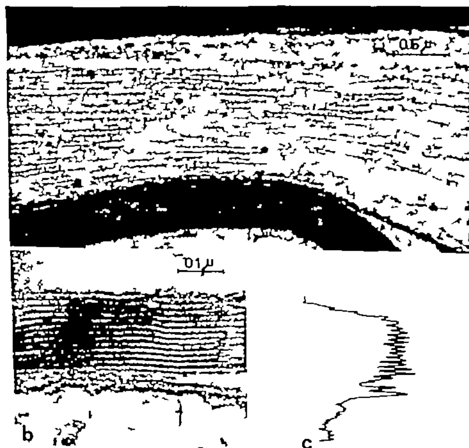


Fig. 15 The plasma membrane of myelinated fiber in the osseous spiral lamina does not show true precipitate but only an increased electron density (33,000 ×) *b* (120,000 ×). This is demonstrated by the densitometric trace in c arrow in *b* shows direction. Chinchilla, glutaraldehyde fixed material.

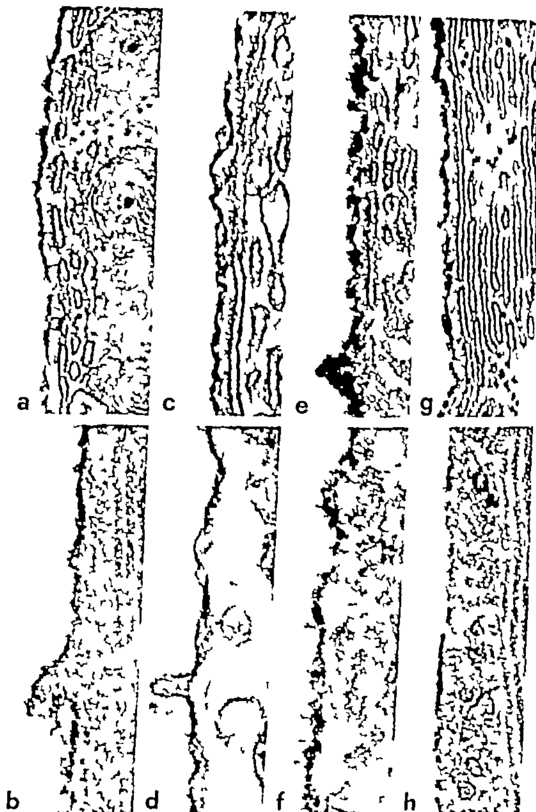


Fig. 14. F ·c f e t the body f ter ha cell (*a*) d p ll (*b d f h*). In *a* and *b* (incu bat on n p med um). nd *d* (in ubation with t bst te), and *f* (ncubation m presence of BW 84), *e* and *h* (ncubation n presence of iso-OMPA) the same result w r obtained. Chm h lla formaldehyde f ed material (60 000 ×)

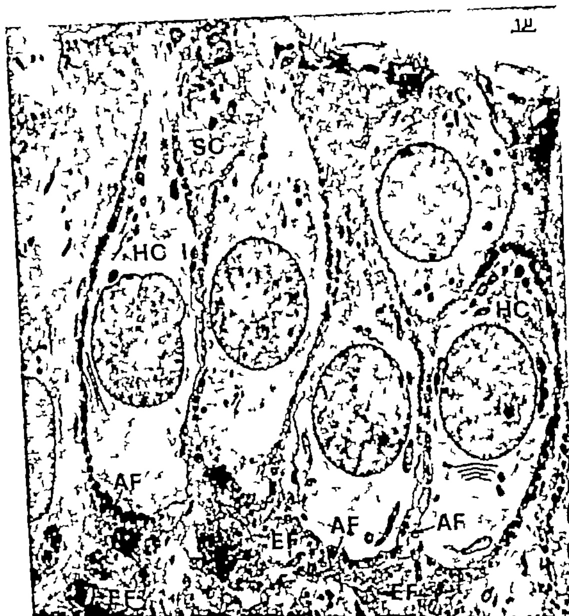


Fig. 17 The reaction product observed in the crista ampullaris is localized on the plasma membrane of the efferent nerve endings (EF) in contact with the outer surface of the afferent nerve chalices (AF). HC I = hair cell type I. SC = supporting cell. 6,000 × Chinchilla formaldehyde fixed material

the presence of 3×10^{-7} M DFP or 1×10^{-5} – 2×10^{-4} iso-OMPA the reaction occurred at all the above mentioned sites

It was concluded that the reaction depe ded on enzymatic activity (control *a*) and no on the activity of non specific reducing group



F g 16. C t la mp lla of chinchilla examined by phase contr t The action product is loc l zed t the level of fferent nerve f bers nd endings EF = efferent f bers o end ngs H = h rs nd *b* (1 100 ×) (1,800 ×) nd *b* f rmaldehyde f ed material glut ...

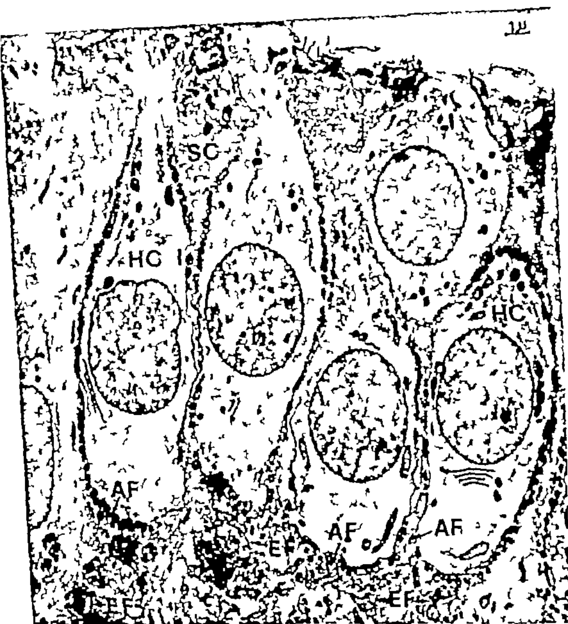
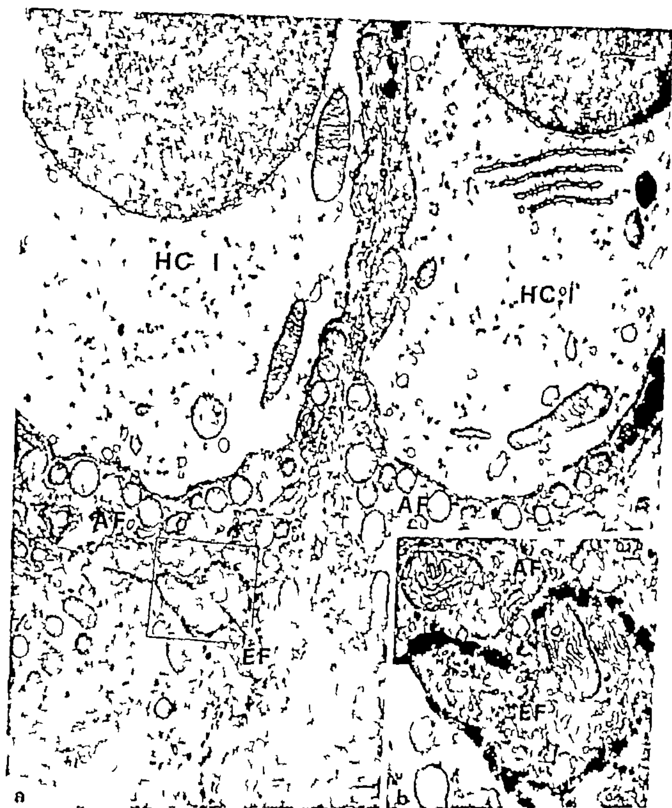


Fig. 17 The reaction product observed in the crista ampullaris localized on the plasma membrane of the efferent nerve endings (EF) in contact with the outer surface of the afferent nerve chalices (AF). HC I = hair cell type I. SC = supporting cell. 6,000 ×. Chinchilla, formaldehyde fixed material.



F ɩ *I* II a ti n p between n n e l rent plasma memb ne of the rent nerve halice (AF) fact f ll the ynaptic gap e end ng (EF) nd the outer surf of the ffe- The re t in pr duct absent in the gap between the ha r cell type I (HC I) and the afferent nerve chalices 14 000 × inset 40 000 × Crista ampullaris of chinchilla f r maldehyde fi ed material.

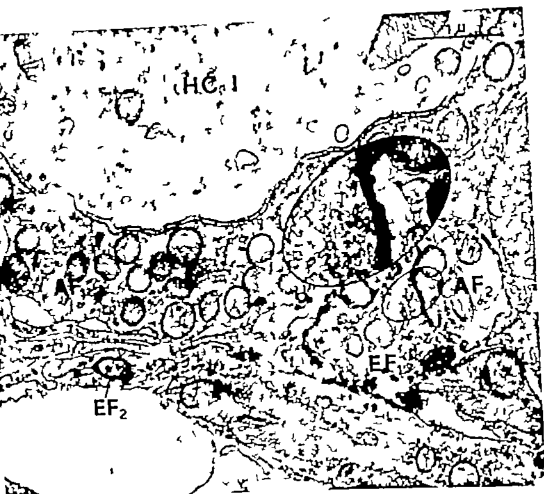


Fig. 19 The small ellipse points to an axodendritic synapse below hair cell type I (HC 1) The larger ellipse shows the same area at higher enlargement AF1 = afferent chalice AF2 = afferent dendrite EF1 = efferent nerve ending EF2 = efferent fiber The reaction product is absent in the gap between the hair cell type I and the afferent nerve chalice 24,000 × inset 60,000 × Chinchilla Formaldehyde fixed material

The controls with inhibitors (control *b*) indicated that the enzymatic activity was due to AChE.

(β) Controls *a* and *b* showed that the plasma membranes of hair cells and pillars and of myelinated fibers were more electron dense than other membranes fine granules were occasionally observed in some areas. It was concluded that this reaction depended on an artefact which at present is unidentifiable.

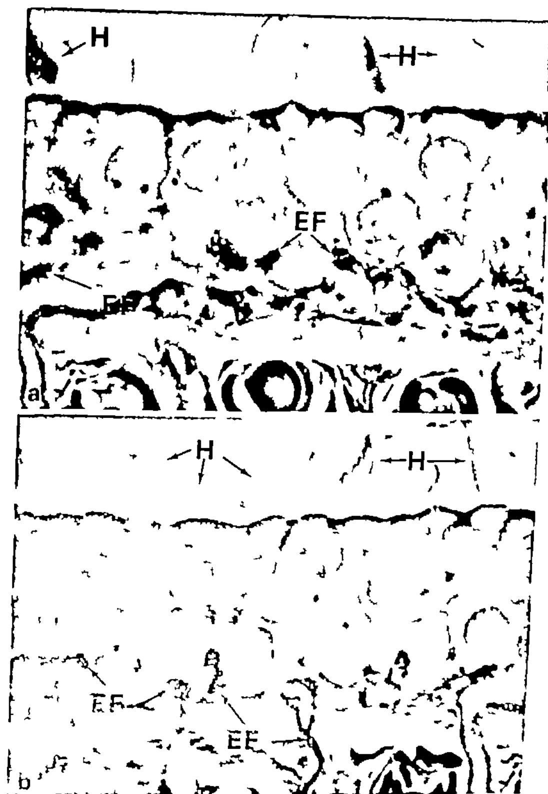
Freezing and thawing (controls *d* and *e*) caused remarkable morphological alterations. However the localization of enzymatic activity did not appear changed neither could different localizations be identified from those previously described



Fig 20 Crista ampullaris of chinchilla 4000 × glutaraldehyde fixed material The reaction product is localized on the plasma membrane of an efferent nerve ending (EF) at the base of a hair cell type II (HC II). The inset shows the outlined area at higher enlargement (44 000 ×). The subsynaptic cistern (SS) does not contain the reaction product



Fig. 21 Crista ampullaris of chinchilla
a Intraepithelial efferent fiber (EF) with evident positive reaction. BM = basement membrane. N = nucleus of supporting cell. 60 000 ×. Formaldehyde fixed material.
b Below the basement membrane the reaction product is localized on the non-myelinated nerve fibers (NM). MY = myelinated nerve fibers. SC = Schwann cell. 30,000 ×. Glutaraldehyde fixed material.



F Utr l l h la xamined by phase H = hairs and *b* (1 600 ×) Formaldehyde f ed n t The r duct i l x l zed t th material. level f eff t n f bers nd endings (EF)

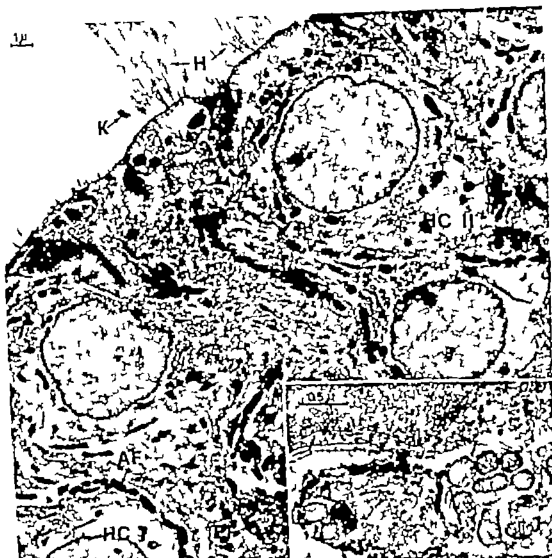
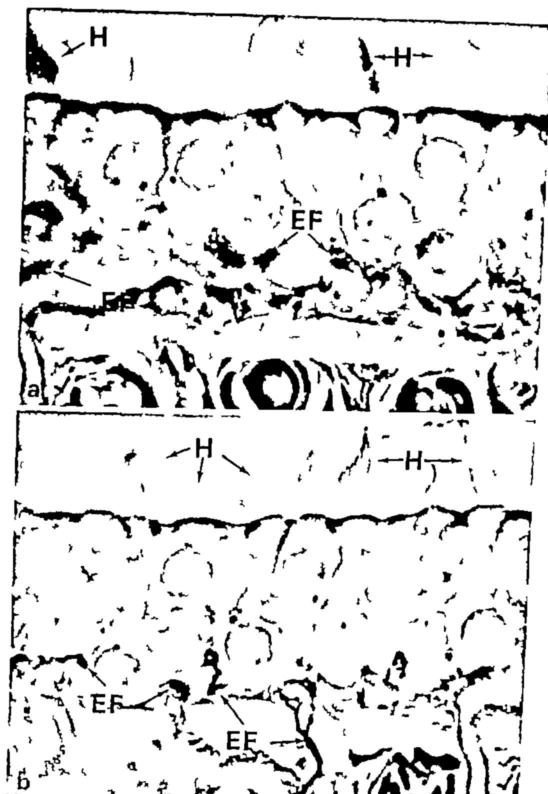


Fig. 23. In the utricle the reaction product is localized on the plasma membrane of the afferent nerve endings (EF). AF = afferent nerve chalice. H = hairs. K = kinocilium. HC I = hair cell type I. HC II = hair cell type II. 6,000 ×. The inset (26,000 ×) shows an axodendritic synapse. Chinchilla. Formaldehyde fixed material.



I Utr l t h an lla xam ned by phase H = ha rs nd *b* (1 600 ×) Formaldehyde f xed nt t The react on product loc lized t the material le el f efferent nerve f bers and end ngs (EF)

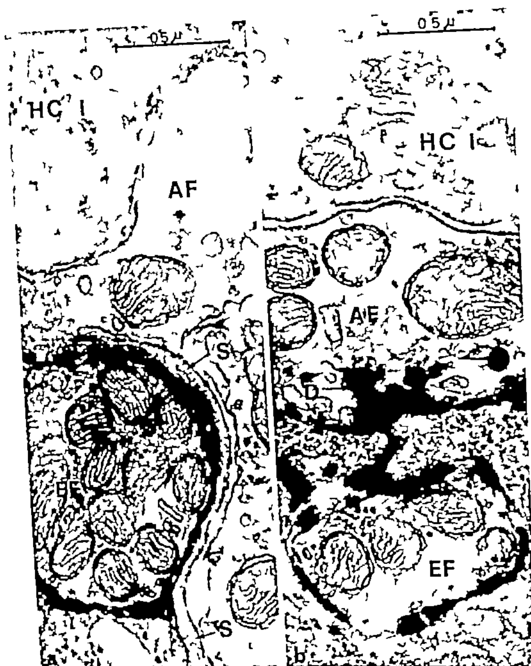


Fig. 25 a and b. Utricle of chinchilla. 60,000 ×. Formaldehyde fixed material. HC I = hair cell type I. AF = afferent nerve chalice. S = supporting cell. The enzymatic activity is localized on the plasma membrane of efferent nerve fibers or endings (EF). Sometimes diffusion phenomena can be observed (D).



Fig 24 Utricle of [illegible] 4000 × Formaldehyde fixed mate ial

a The reaction pr duct is localized on the plasma membrane of efferent nerve end ng (EF) at the base of a ha r ell type II (HC II) The fferent nerve end ngs (AF) do not how the re t on pr duct SY = ynaptic bar

b The re ction product f ll the gap between n fferent nerve end ng (EF) nd the plasma membrane of ff rent nerv [illegible] type I S = support

Discussion and interpretation of the results

The results of the present study generally agree with those obtained by SCHUKNECHT et al. (1959), VINNIKOV and TITOVA (1961 1962, 1963 1964) and DOHLMAN et al. (1958) with the light microscope in the cat, guinea pig and pigeon and by KANEKO (1968) and KANEKO and DALY (1968) with the electron microscope in the guinea pig.

The satisfactory preservation of the tissue and the relative fine distribution of the reaction product give course for discussion on the problems of (1) AChE localization and (2) possible significance of AChE activity in the inner ear

1. AChE localization

The localization of AChE activity at the electron microscope can be influenced by (a) fixation, (b) displacement of intermediate products and/or of the end product of the histochemical reaction and ( ) the steps following the histochemical reaction (e.g. rinsing, post fixation, dehydration and embedding) All these factors can give an incorrect interpretation of the results From this point of view the technique of KARNOVSKY and ROOTS (1964) has been thoroughly examined by the authors (KARNOVSKY 1964) and also by BLOOM and BARRNETT (1966). NOVIKOFF et al (1966) ERÄNKÖ et al (1967), ROBINSON and BELL (1967), ROBINSON (1969). Other histochemical methods that allow the localization of the same enzyme have been even more critically controlled (e.g. BROWN, 1961 BARRNETT 1962 BRZIN et al 1966 DAVIS and KOELLE, 1967). We have considered the results of these last studies because most of the techniques, includ ing those used by KARNOVSKY and ROOTS (1964) derive from the KOELLE and FRIEDENWALD's (1949) method

a) Fixation

It is well known that ChE are at least in part, firmly bounded to the cell structures (COUTEAUX, 1958 BARRNETT 1962 KARNOVSKY 1964 and others) and therefore not easily dif fusible However a fixation is advised for the following reasons to (α) assure the mor phological preservation of structures (ERÄNKÖ et al. 1967) (β) reduce enzymatic activity in the areas in which it is present in an excessive amount and obtain a clearer localization of the end product of the reaction (COUTEAUX, 1958), (γ) make the penetration of the com ponents of the incubation medium easier (BRZIN et al. 1966)

In the course of this study we used two fixatives, formaldehyde and glutaraldehyde. Formaldehyde inhibits ChE activity in different amounts according to the organ and type of animal (TAXI 1957). Inhibition has also been seen with glutaraldehyde (SABATINI et al 1963 BLOOM and BARRNETT 1966 NOVIKOFF et al 1966 and others). In our material formal dehyde gave a less evident (by judging the intensity of the reaction) inhibition of AChE activity The results were the same as those obtained by other authors on the same (KANEKO and DALY 1968) and on different materials



F g 26 Bel w the basement membrane of the utricle the react on product is localized on the non-myel nated nerve f bers (NM) MY = myelinated nerve fibers SC = Schwann cell 60,000 × Chinch lla F rmaldehyde f ed material

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(e.g. SHIMIZU and ISHII 1966; TERÄVÄINEN 1969 b). However BLOOM and BARRNETT (1966) noted that formaldehyde inhibits more or less the same as glutaraldehyde. The different results can be explained by various sources of aldehydes with different concentrations of the fixative and also with different fixation times.

We fixed the material *in vivo* using the perilymphatic perfusion method advised in the studies of the inner ear to obtain better morphological results (IURATO and DE PETRIS 1967). In other material fixation *in vivo* was advised to reduce or abolish a possible displacement of the enzyme (ROBINSON and BELL, 1967; ROBINSON 1969).

b) Diffusion of the intermediate products and/or of the end product of the reaction

KARNOVSKY's technique was chosen by us because it is simpler and adapted to the study of the inner ear. Moreover it does not give artefacts caused by « metallophilia », i.e. a bound between some tissue components (probably acid mucopolysaccharides) and metal ions (ZENKER 1969; LANGLEY 1969).

The end product of the reaction is insoluble in water and in the reagents used for embedding (KARNOVSKY 1964). NOVIKOFF et al. (1966) however did not exclude the possibility of its own displacement, although this only happens in unfixed materials. ROBINSON (1969) and TERÄVÄINEN (1969 a) thought that thiocholine released by enzymatic activity was diffusible. This diffusion should happen if the speed of substrate hydrolysis is greater than the speed of capture, that is to say in the places where the enzymatic activity is higher. In such a case one could have a "false negative reaction" (in the place of the true enzymatic activity) and a "false positive reaction" (where the thiocholine is diffused). In order to reduce or eliminate the diffusion it was suggested to partially inhibit enzymatic activity by a more or less prolonged fixation (BARRNETT 1962; ERÄNKÖ et al. 1967; SHIMIZU and ISHII 1966), by unfavourable pH (KARNOVSKY and ROOTS 1964), by lowering of temperature, duration of incubation (KARNOVSKY 1964), or by adding an inhibitor in weak concentration to the incubation medium (BARRNETT 1962). These devices give the best conditions to the histochemical localization of the enzymatic activity. Not knowing the place in the inner ear in which ChE activity is located in ... and above all how much could be found, the time of fixation was maintained constant and we varied the duration of the incubation which was carried out on ice. In this way diffusion was easily limited and identified.

c) Steps following the histochemical reaction

According to KARNOVSKY and ROOTS (1964) and KARNOVSKY (1964) these steps do not produce artefacts. KARNOVSKY (1964) said that counterstaining with an alcaline Pb solution does not influence the end product of the reaction. On the contrary we observed that the staining of the thin sections using a Pb citrate solution according to REYNOLDS (1963) can remove the end product of the reaction after 5 min. This artefact is very coarse and easily identifiable. It can be avoided if a quick staining method is used like that proposed by VENABLE and COGGESHALL (1955) which has lately been used by us.

On the basis of the criteria discussed in (a), (b) and (c) the results of the histochemical reaction obtained in the different areas of the membranous labyrinth can be critically examined and interpreted.

In the inner spiral bundle, where large Cu ferrocyanid crystals were observed, some axons were completely surrounded by the reaction product, some only partially and others not at all (Fig. 4). It could be thought that for the latter the reaction was negative. However serial sections demonstrated that the reaction was present where before it was

absent and vice versa (Fig. 5 *a, b*). The negative reaction of some axons probably depends on the large volume of crystals formed during incubation. The observation of serial sections therefore permits us to exclude a "false negative reaction".

Sometimes the gap between the small afferent nerve endings and the outer hair cells showed the end product of the reaction (Fig. 8). This could be interpreted as the result of a diffusion phenomenon (which produces a "false positive reaction") for the following reasons. (α) The reaction product was always located in larger quantities in the part of the gap closer to an efferent synapse. (β) In the middle and upper turns of the cochlea where the large efferent endings are less numerous, the small afferent nerve endings were usually completely negative (Fig. 10). (γ) The large efferent nerve endings showed an intense deposition of Cu-ferrocyanide not only in the synaptic gap but also on the whole plasma membrane besides the synaptic region. Instead, in the small afferent nerve endings the reaction product, when present, was only deposited in the part of the synaptic gap close to an efferent synapse. The present interpretation agrees with that of KANEKO and DALY (1968). The precipitate that was sometimes observed in the gap between the hair cell type II and the afferent endings (Fig. 20) could be interpreted in the same way.

The problem whether these "false localizations" depended on the diffusion of the reaction product or of the enzyme cannot be solved at present because the reaction product obtained by us is still too coarse. The problem could be resolved after having determined if the reaction product is caused by an enzymatic activity really present in the synaptic gap on both pre- and post-synaptic membranes or only on the pre- or on the post-synaptic membrane. The research carried out by DE LORENZO et al. (1969) and by TERAVAINEN (1967, 1969 *a, b*) demonstrated that a precise localization depends on many factors, which are particulary difficult to control in the inner ear.

The enlargements of the synaptic gap which were found in the efferent synapses, particularly at the level of the active zones, were probably due to the large size of the reaction product (Fig. 9–11). This interpretation rests on the following facts. (α) The synaptic gap is constant in width in the specimens not treated with histochemical methods. (β) In this area the enzymatic activity was high and the reaction product was formed in a large amount after only 30 min of incubation on ice in spite of glutaraldehyde fixation. (γ) The enlarged tracts of the synaptic gap always appeared completely filled by the reaction product.

The junction between the hair cells type I and the nerve chalices was always free of the reaction product (Fig. 18, 19, 24 *b*, 25). At this level a finely granular intercellular material is interposed in a regular manner between the plasma membrane of the hair cell and the nerve chalice (C. A. SMITH, 1967 c; HAMILTON, 1968). In order to be certain that the absence of AChE activity was not due to an inadequate penetration of the medium in this gap, particulary when the material was incubated *in toto*, we made some trials on frozen and thawed material and on sections cut using the freezing microtome. By these techniques severe morphological alterations were found in the neuroepithelium. However, this procedure did not produce a different localization of the enzymatic activity. We were therefore confident that the lack of enzymatic activity at the hair cell-chalice junction did not depend on an inadequate penetration of the medium.

As previously mentioned in the introduction, AChE activity was also observed in the cytoplasm of the hair cells with the aid of the light microscope (DEL BO and CONTI, 1961; VINNIKOV and TITOVA, 1961, 1962, 1963, 1964). However, in the present investigation although a careful examination of the electron micrographs, the reaction product was never found inside the hair cells nor in the nerve endings. It is a well known fact that with the aid of the electron microscope AChE activity

was observed in many cytoplasmic organelles of other tissues e g the synaptic vesicles, the endoplasmic reticulum and *sometimes the* Golgi apparatus (BARRNETT 1962 MILEDI *1964* BLOOM and BARRNETT *1966*) the neurotubules (SHIMIZU and ISHII 1966 DE LORENZO et al 1969) and the subsynaptic cistern (BRZIN et al. *1966*)

The discrepancy could depend on the difficulty of the components of the incubation medium to penetrate into the tissues (BELL, *1966* ERÄNKÖ *et al 1967* DAVIS *and* KOELLE 1967), when incubated *in toto* In this connection it may be recalled that many authors (e g COUTEAUX, 1958 KOELLE and FOROGLOU KERAMEOS 1965 KOELLE and GROMADZKI *1966) suggested a* pre incubation in a medium without substrate to obtain the penetration and the equilibrium of all the reagents inside the specimen before the substrate arrives at the active points. NOMURA (1966) on the other *hand* demonstrated in the inner ear with the light microscope a different localization of DPNH-diaphorase activity depending on the degree of the *penetration of the* incubation medium The penetration can be made easier by freezing and thawing the specimen resulting in the breaking of the plasma membranes BELL (1966) obtained a similar result with the light *microscope in the* central nervous system using KARNOVSKY and ROOTS technique for ChE

In order to be certain that the absence of AChE activity within the hair cells and in the nerve endings was not due to an inadequate *penetration of the medium we made some* trials on frozen and thawed material (control *f*) By freezing and thawing, severe morphological alterations were found in the organ of CORTI and in the vestibular sensory areas at *the electron microscopical* level However this procedure did not produce a different localization of the *enzymatic* activity It is evident that fixation was sufficient to diminish the barrier to diffusion of the incubation medium (BRZIN et al. 1966) which in our material consisted of only a few rows of cells.

The weak reaction we noted on the free surface of the hair cells could depend on (α) the inhibition *of the enzymatic activity by the* fixative or (β) an unspecific staining It must *be recalled that the* precipitate was only observed after fixation in formaldehyde and not in glutaraldehyde Since formaldehyde inhibits *less than glutaraldehyde ChE activity* one could conclude that the precipitate was due to a specific enzymatic reaction. Instead the controls indicated that the reaction was unspecific. Infact the precipitate was observed in *the material incubated* without substrate and in the presence of inhibitors. KANEKO and DALY (1968) noted a lesser quantity of precipitate on the cuticle and hairs of outer hair cells in material incubated without substrate and concluded *that* the reaction was due to AChE activity On the other hand the results obtained by us demonstrated that the precipitate was unspecific (Fig. 13 and 14) and was not due to enzymatic activity The reason for *this artefact has not yet been identified.*

A positive reaction was observed *on the* plasma membrane of myelinated nerve fibers by LEWIS and SHUTE (1966) NOVIKOFF et al. (1966) SCHLAEPFER and TORACK (1966) BRZIN et al. (1966) BRZIN and DETTBARN (1967). *SCHLAEPFER (1968) CSILLIK et al* (1968), DI LORENZO et al (1969) and other authors. UCHIZONO et al (1967) observed the same on the cochlear myelinated fibers. In our material the incubation medium penetrated with difficulty *into the* cochlear nerve fibers because the structure of the myelin sheath was well preserved This could explain *the weak reaction* we noted However ELFVIN (1961) and other authors (e g MALHOTRA and VAN HARREVELD, 1965) observed that the axolemma was thicker than the plasma membrane of Schwann cells *In our material* the densitometric trace (Fig 15) confirmed these observations and also showed that *the plasma membrane of myelinated* nerve

fibers was thicker than the plasma membrane of Schwann cells and major dense layers of myelin sheath. Since the densitometric trace of controls was similar, we concluded that the increased density was not due to the histochemical reaction.

Finally, after fixation in glutaraldehyde electron dense granules of 500 Å could be observed spread at random on all the cell structures. They were not present in the material incubated without substrate (control *a*); they were very numerous when the material was incubated in a stale medium (control *c*). In agreement with ROBINSON (1969) we think that these granules are unspecific and produced in the incubation medium by spontaneous hydrolysis of the substrate. ROBINSON avoided this inconvenience by a short incubation in a fresh medium, while our experiments using the same method were unsuccessful.

Summarizing, in the organ of CORTI and in the vestibular sensory areas AChE activity seems to be an exclusive property of efferent fibers, efferent nerve endings and synaptic enlargements along efferent presynaptic fibers.

2. Possible significance

The problem of the identification of the neurohumoral transmitter in the efferent olivocochlear bundle (OCB) is still open to discussion. On the basis of its AChE activity KOELLE (1955), CHURCHILL et al. (1956), SCHUKNECHT et al. (1959), ROSSI and CORTESINA (196) and SHUTE and LEWIS (1963) considered the efferent OCB as cholinergic. However, according to some more recent investigations the histochemical localization of AChE in an axon does not automatically identify that axon as cholinergic in the accepted sense (discussion in ROBINSON and BELL, 1967). As pointed out by ECCLES (1964), besides the histochemical one several other criteria have to be satisfied before one can have full confidence in identifying a substance as the synaptic transmitter of a system of nerve fibers. Particularly important are the neuropharmacological tests. Unfortunately in the case of the organ of CORTI the pharmacological tests have given contradictory results.

GISSELSSON (1950, 1960) applied acetylcholine (ACh) electrophoretically to the vicinity of the hair cells and found an increase of the cochlear microphonics (CM) amplitude. After intra-arterial injection of ACh, the action potentials (AP) of the auditory nerve rapidly disappeared but it was uncertain whether this was due to a specific effect or to a generalized action on the animal. A similar effect, namely inhibition of AP and augmentation of CM was obtained by FEX (1959, 1962) and by DESMEDT and MONACO (1960, 1961) with the electrical stimulation of the crossed efferent fibers. Thus GISSELSSON (1960) suggested that ACh was the transmitter substance of the crossed OCB fibers.

On the contrary KATSUKI et al. (1965), TANAKA and KATSUKI (1966) obtained opposite results, namely decrease of CM response and no effect on AP by applying ACh iontophoretically in the vicinity of the cochlear hair cells. Moreover, the local application of *d*-tubocurarine (DTC) in the vicinity of the hair cells does not influence the inhibition of AP caused by electrical stimulation of the OCB at the floor of fourth ventricle. These results suggest that the efferent fibers to the cochlea are noncholinergic.

Unfortunately neither GISSELSSON nor KATSUKI mentioned the concentrations of ACh they used. FEX (1968) estimated that the amount of ACh applied by GISSELSSON could be lower by a factor of 1,000 or more than the amount that KATSUKI et al. applied. Also the description of the DTC experiments made by TANAKA and KATSUKI is not sufficiently detailed. FEX (1968) applied DTC to the organ of CORTI of the cat using artificial perilymph to which 0.7×10^{-4} gr/ml of DTC chloride had

was observed in many cytoplasmic organelles of other tissues e g. the synaptic vesicles the endoplasmic reticulum and sometimes the Golgi apparatus (BARRNETT 1962 MILEDI 1964 BLOOM and BARRNETT 1966) the neurotubules (SHIMIZU and ISHII 1966 DE LORENZO et al 1969) and the subsynaptic cistern (BRZIN et al. 1966)

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TURKU 1971

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X-ray crystallographic study in my investigation was carried out at the Institute of Physics, Turku University Head of this institute Professor Martti Kantola, Ph D kindly offered his apparatus to my disposal and showed unfailing interest for my work. In addition, I am thankful for his sound criticism.

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For good cooperation I am indebted to Mr Erkki Häsänen Lic.Technol. His help made it possible to carry out the mineral analyses of bone at the Reactor Laboratory of the Department of Technical Physics Technical University Otaniemi.

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CONTENTS

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I Introduction

Although probably *the first reports of otosclerotic* changes were published as early as in 1735 by Valsalva, it took almost 200 years before the histopathologic phenomena of this disease were understood more carefully. It is true that Politzer in 1894 described the otosclerotic process more in details, but only the twentieth century brought more defined information on the development of this disease. Yet the final answer to the question of the initiating factor of the process, which in its time leads to formation of abnormal ossification foci is still missing.

It has been discussed for a long time whether otosclerosis is a primary disease of the bony labyrinthine capsule or a local manifestation of a more generalized disease. The van der Hoeve syndrome has often been an object *of investigation in this connection.* In the same way the investigators have tried to found a connection between otosclerosis and osteogenesis imperfecta. Morrison & Bundley (1970) write however that there is no evidence to relate the two diseases in the language of clinical genetics. Bentzen (1966) on his behalf noted not infrequently different ectodermal and mesodermal abnormalities in his otosclerotic patients. This can be considered to mean a more generalized process.

Some experimental studies of otosclerosis have enriched the discussion on possible etiologic factors. Angeluscheff (1953) reports changes in the bone after ultrasonic exposure. He found similarities with the otosclerotic process and refers to prevalence of ultrasounds in our daily environment. Kristensen & Jorgensen (1967) examined the temporal bones of 165 patients and noted a relationship between irradiation and otosclerotic changes. Mendoza et al. (1969) irradiated labyrinthine capsules of dogs by x-rays and found foci resembling of otosclerosis. It is questionable whether these changes observed have any *connection with* the proper otosclerotic process.

According to Altmann (1962) the etiologic factors of otosclerosis can be constitutional, local or activating, and it is most likely that several factors exist that cause the pathologic process. According to Frost (1962) the primary etiologic factor is some change in the cement substance of the bony labyrinthine capsule. When we remember on the other hand that the organic component of the bone play a decisive role in initiating and developing the mineralizing process a change in the bone cement substance may *cause different disorders in the inorganic* component of the otosclerotic bone. Röckert et al. (1965) write: "One of the regions where basic information is lacking is the mineralizing process in otosclerosis. Among the numerous investigations on otosclerosis, there are only few which deal with the inorganic side of the process. This may be due to the small number of available methods. X-ray diffraction methods, electron microscopy, soft roentgen contact microradiography and, in recent years some other methods as well have given a chance to deepen our knowledge of the inorganic component of the bone. It is probable *that elucidation* of the inorganic component of the otosclerotic process does not give the final answer to the question of the initiating factor of otosclerosis, but the investigation may *give a hint at the direction* where the real cause should be sought.

II Review of the literature

A. Some aspects of ossification and bone crystals

Bone formation begins with the mesenchymal cells gradually changing into osteoblasts which are in their turn able to produce tropocollagen molecules and cement substance (Ham 1965) Although the fibroblasts in several tissues are able to form collagen osteoblasts only are able to produce cement substance typical of bone Tropocollagen molecules are of ultramicroscopic dimension and they have a constant chemical composition and fixed molecular weight Fibrils of different lengths and thicknesses are formed of tropocollagen by polymerization (Frost 1962)

Osteoblasts which form fibrous bone have chemical activity which differs in many respects from the activity of the osteoblasts which form lamellar bone Fibrous bone is formed very rapidly but it is structurally inferior Regular structure is lacking in it and its lacunae are not oriented. Normally fibrous bone is rapidly replaced by lamellar bone and human bones are almost exclusively lamellar Fibrous bone is most often imperfectly mineralized Frost (1962) has noted that otosclerotic bone very much resembles the fibrous bone that is seen in connection with osteomyelitis and healing fractures

The cause why the collagen of bone can alone start the mineralization process is still mostly unknown Neuman (1950) has suggested that there is an organic inhibitor which can be destroyed by alkaline phosphatase during calcification This inhibitor may be a polyphosphate and Fleisch & Neuman (1960) have observed its presence in serum ultrafiltrate and urine.

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means that these fragments gradually grow into clearly defined crystals. In the nucleation phenomenon, the nuclei probably grow in the way that new molecules atoms and ions gradually come into the nucleus (Glimcher 1959) Recrystallization means the growth of large crystals at the expense of smaller ones.

The shape, size and orientation of bone crystals can be examined by virtue of x-ray diffraction. Due to the relatively small size of bone crystals the diffraction lines are, however broad and somewhat indistinct causing difficulties in estimating the results. For the same reason it is not easy to carry out a crystallographic examination between different suggested bone structures. The exact composition of the bone is still discussed. Yet, a generally accepted view is that the physical unit of bone is apatite, more closely hydroxyapatite crystal (Carlstrom & Glas 1959 Glimcher 1959 Engström 1960) The chemical structure of the unit cell represents the formula $Ca_{10}(PO_4)_6(OH)_2$ (Fig. 1)

Apparently collagen fibrils primarily determine the size and shape of bone crystals. Organic molecules influence the growth of inorganic crystals (Seifert 1953) Termine & Posner (1967) have shown that big part of bone minerals are in noncrystalline amorphous phase. It has been suggested that bone crystals are large hexagonal plates 500 x 250 x 100 $Å^2$ in size (Robinson 1952) but the most often accepted view is that bone crystals are needle-shaped particles with a thickness of 15 to 30 Å and length of 200 to 400 Å (Glimcher 1959 Frost 1962) Nevertheless a considerable variation in the length of the crystals is possible (Eanes & Posner 1970)

In his monograph, Aho (1966) has presented a general survey of appearance location in respect to collagen fibrils and variations in the shape of bone crystals. Robinson & Cameron (1956) have showed electron microscopically that the long axis

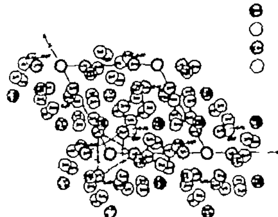


Fig. 1 The hydroxyapatite structure projected onto the a, b plane. The solid lines drawn between the hydroxyl positions outline the unit cell edges. The centers of the open circles mark the x, y positions of the atoms. The z parameters are the numbers written within the circles. (From Eanes & Posner 1970)

of bone crystals is parallel to the fibrils, but in the crystallizing cartilage crystals are not oriented. Similarly Clark & Iball (1957) have pointed out that crystals in embryonic bone are not oriented. Crystal orientation varies not only from bone to bone but also in different locations of the same bone. Collagen fibrils are hardly either oriented in embryonic bone (Glimcher 1959) The fact that the crystals are not oriented is not due to the crystals themselves, but the indefinite location of collagen fibrils. The most important reason for orientation of inorganic bone crystals is the asymmetric growth of small crystals within the collagen fibrils, between tightly packed, longitudinally oriented chains of macromolecules (protofibrils)

B. Arrangement of collagen fibrils and bone crystals in the auditory ossicles and otosclerotic focus

1 The auditory ossicles

Bast & Anson (1949) rather carefully described the general structure of the auditory

II Review of the literature

A. Some aspects of ossification and bone crystals

Bone formation begins with the mesenchymal cells gradually changing into osteoblasts which are in their turn able to produce tropocollagen molecules and cement substance (Ham 1965) Although the fibroblasts in several tissues are able to form collagen osteoblasts only are able to produce cement substance typical of bone Tropocollagen molecules are of ultramicroscopic dimension and they have a constant chemical composition and fixed molecular weight Fibrils of different lengths and thicknesses are formed of tropocollagen by polymerization (Frost 1962)

Osteoblasts which form fibrous bone have chemical activity which differs in many respects from the activity of the osteoblasts which form lamellar bone Fibrous bone is formed very rapidly but it is structurally inferior Regular structure is lacking in it and its lacunae are not oriented. Normally fibrous bone is rapidly replaced by lamellar bone and human bones are almost exclusively lamellar Fibrous bone is most often imperfectly mineralized Frost (1962) has noted that otosclerotic bone very much resembles the fibrous bone that is seen in connection with osteomyelitis and healing fractures

The cause why the collagen of bone can alone start the mineralization process is still mostly unknown Neuman (1950) has suggested that there is an organic inhibitor which can be destroyed by alkaline phosphatase during calcification This inhibitor may be a polyphosphate and Fleisch & Neuman (1960) have observed its presence in serum ultrafiltrate and urine

Calcification theories can be divided into three classes (1) those which propose mechanisms that would raise locally the $(Ca)x(PO_4)$ solution product to levels at which apatite would precipitate spontaneously (2) those which propose mechanisms that would create nucleating sites, or remove barriers to these sites, at local calcifying areas and (3) those theories which propose that apatite does not precipitate directly from serum but that a less basic calcium phosphate salt is formed initially which then hydrolyzes into apatite (Eanes & Posner 1970)

Weidmann (1963) thinks that mineralization of bone is a complicated and multiphase event There are three different possibilities that can initiate mineralization (1) collagen has a special function in the initiation of calcification (2) a combination of chondroitin sulphate and collagen has significance in providing the initial stimulus for mineralization (3) phosporylated collagen or phosphorylated component in ground substance acts as the initiating factor

According to Taves (1965) the most popular hypothesis for the mechanisms of normal calcification — diffusion of all ions and nucleation by the matrix — has been shown to be insufficient More evidence is needed to establish the suggestion that collagen nucleate at all Considerations of some type of pump mechanism or booster is according to Taves potentially useful.

Crystallization which means formation of inorganic crystals in a place where they have not existed previously represents a phase change This physical change in state can be divided into crystal nucleation which means formation of the initial fragments of the new phase and crystal growth which

means that these fragments gradually grow into clearly defined crystals. In the nucleation phenomenon, the nuclei probably grow in the way that new molecules atoms and ions gradually come into the nucleus (Glimcher 1959) Recrystallization means the growth of large crystals at the expense of smaller ones.

The shape, size, and orientation of bone crystals can be examined by virtue of x-ray diffraction. Due to the relatively small size of bone crystals the diffraction lines are, however broad and somewhat indistinct causing difficulties in estimating the results. For the same reason it is not easy to carry out a crystallographic examination between different suggested bone structures. The exact composition of the bone is still discussed. Yet a generally accepted view is that the physical unit of bone is apatite more closely hydroxyapatite crystal (Carlström & Glas 1959 Glimcher 1959 Engström 1960) The chemical structure of the unit cell represents the formula $Ca_{10}(PO_4)_6(OH)_2$ (Fig. 1)

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B. Arrangement of collagen fibrils and bone crystals in the auditory ossicles and otosclerotic focus

1 The auditory ossicles

Bast & Anson (1949) rather carefully described the general structure of the auditory

ossicles On the other hand the periodic structure and arrangement of collagen fibrils are not presented in their investiga tion. According to them the stapedial foot plate is only partly osseous and partly cartilaginous Wustrow (1956) made a series of section samples of the auditory ossicles He found that irregularly located fibrils are however arranged by a certain princi ple. Differences in histologic pictures are due to different cut surfaces of fibril ag- gregations Chevance (1961) examined the arrangement of collagen fibrils in the audi- tory ossicles of man calf and Guinea pig and observed that collagen fibrils in the malleus incus and the stapedial crura are arranged in bundles A more irregular ar rangement was found in the stapedial foot plate which in Chevance s opinion was due to the fact that the stapedial footplate originates from the labyrinthine capsule Reydon & Smith (1968) examined the foot plate of the normal stapes by light and electron microscope and noticed remarkable structural variability in the order of collagen fibrils A lamellar arrangement of some of the collagen fibrils resembling the haversian system was present

There are very few references to crystallo- graphy of the auditory ossicles in the literature Meurman et al. (1967 1968) examined the crystalline structure of normal auditory ossicles and otosclerotic foci by the Debye-Scherrer method They noted that x ray crystallographic characteristics of normal auditory ossicles closely corre sponded to those of normal human bone A conclusion could thus be drawn that the crystalline structure of normal auditory ossicles does not differ from that of normal human bone

2 *Otosclerotic focus*

Weber (1931) examined the arrangement of collagen fibrils in otosclerotic foci in polar ized light and found that the fibrils were broken abgeschnitten" in the edge of the focus. In addition there were concavities in the fibrils. According to Weber the fibrils in the focus clearly differ from those outside the focus.

In their investigation on otosclerotic foci at different stages Chevance et al. (1961a) found that at the destruction phase collagen fibrils were broken as if they were cut with a knife and that this collagenolysis is a purely biochemical phenomenon because no cellular reaction is observed. In the electron microscope Chevance (1962) was able to distinguish different collagen changes at a great magnification

Müller (1964) thinks that it is possible to conclude upon the maturity of a otosclerotic focus from the form and arrangement of bone fibrils In an inactive focus the ar rangement of the fibrils is parallel and circulatory In an active phase on the contrary the arrangement form and thick ness of the fibrils vary a great deal. In some cases no fibrils can be seen. In their electron microscopic investigations Reydon & Smith (1968) found that in the central parts of the marrow spaces no connective tissue fibers were present On the other hand a great number of osteoblasts and their cytoplasmic processes were seen but no osteoclasts at all In the inactive parts collagen fibrils were mainly irregularly ar ranged

The crystalline structure of otosclerotic bone has been examined very little Meur man et al (1967 1968) used x ray diffraction methods and examined 200 otosclerotic foci or otosclerotic footplates in all In most of the samples the crystalline structure of otosclerotic bone was identical with that of normal human bone In one part of the samples no clear crystalline structure was distinguished at all and in another minor part a structure different from the crystal- line structure of normal hydroxyapatite was noted The authors suggested that these pathologic crystalline structures might

represent a mature inactive otosclerotic focus.

Frank et al. (1968) elucidated crystallography of otosclerotic foci by using electron microscopy and electronic diffraction in their investigations. In their diffraction photographs a part of the apatite lines had disappeared, and in addition they were broad and indistinct. The authors concluded on the basis of this that the crystals were small. In the electron microscope they were able to see the reduced size of the crystals.

C. Histochemical structure of the otosclerotic focus

To-day a great deal is known about what happens in the otosclerotic focus but very little about the initiating factor of this process. When examining otosclerosis by electron microscopy Chevance et al. (1969) noted that there were microfoci between the focus and normal tissue demonstrable only in the electron microscope. The focus appears to enlarge by fusion of these microfoci. In Gussen's (1968) opinion formation and spreading of otosclerotic bone are two quite different processes. The former is new bone formation within fibrous tissue and the latter invasive bone spreading.

Keleman & Linthicum (1969) in their turn think that every otosclerotic focus contains a spongiotic and sclerotic portion, which can always be sharply divided from each other. They did not find any cases of so-called burned out foci in old patients or of preotosclerosis around the advancing focus.

Some investigators suggest that decalcification of bone in otosclerotic process proceeds without osteoclasts (Lempert & Wolff 1949, Wolff & Lempert 1965, Chevance et al. 1969) whereas others consider osteoclasts a very important factor in this process (Weber 1960). Insufficiency of osteoblasts has been taken for the primary factor of otosclerosis (Hall & Oglivie 1961, Hoogland 1962, Hall & Oglivie 1964). Since the foci usually spread along the vascular spaces (Hall 1952, Wolff & Lempert 1965) it has been suggested that vascular changes participate in the genesis of decalcification (Lempert & Wolff 1949). Mendoza & Rius (1966) found obliteration of a large number of arteries in otosclerotic bone and Riiedi (1969) noted abnormal vascular connections between the membranous labyrinth and focus. Chevance et al. (1969) observed by electron microscopy that the endothelial cells of capillary network of the focus appeared to be normal.

Chevance et al. (1961a) proposed that collagen fibrils and ground substance play a decisive role in the development of otosclerosis. Ardouin & Wegmann (1961) observed that mucopolysaccharides and polysaccharides are disturbed in the otosclerotic focus. Arslan & Ricci (1961) noted deep alterations in the osteomucoid substance too. Ricci (1962) found an abnormal accumulation of mucopolysaccharides in the otosclerotic focus. He suggested that the primary cause of otosclerosis is a change of ground substance. Privara (1966) stated that the reaction of acid mucopolysaccharide in the otosclerotic focus is positive during the stage of breakdown and transformation of bony tissue and negative during the inactive phase.

Wolff & Bellucci (1960) suggested that some enzyme is the initiating factor of decalcification. Later on, efforts has been made to find possible changes in enzyme activities in the otosclerotic focus. Chevance et al. (1961b) noted that there is alkaline phosphatase in the focus at the first phase and during the whole second phase, too until the bone is completely calcified. There after alkaline phosphatase disappears. The enzyme was always located extracellularly except in osteoblasts, where it was intracellular. Ricci (1962) observed as well that the activity of alkaline phosphatase increases in the focus. He found alkaline phosphatase both in osteocytes and ground substance.

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patients and increase in others. Seifert (1937) observed hypercalcemia and hypophosphatemia and increased secretion of calcium in urine.

Fowler (1947) did not see any differences in the calcium and phosphorus contents in investigations with identical twins. In a more extensive investigation of his Fowler (1948a) noted that there was inorganic phosphorus less than 3,5 mg % in 62 % of the men and 66 % of the women. Values of phosphorus were less than 3,5 mg % in two thirds of those whose calcium content was under 9,5 mg % and values of calcium did not reach up to 9,5 mg % in two thirds of those whose phosphorus was less than 3,5 mg %. Fowler considered the changes in the calcium and phosphorus contents general in otosclerotic patients. The reason why these changes were not great was in his opinion the fact that the examinations had been carried out a remarkable time after the onset of the active period of the disease. He thougt that because the lesion of otosclerosis is limited to the petrous bone it cannot cause very great changes in the composition of serum. On the other hand, Riskaer (1949) did not find significant differences in the calcium and phosphorus contents of serum between otosclerotic and control patients. Maurer (1956, 1958) also discussed the significance of calcium and phosphorus values of serum in the genesis of otosclerosis. In a material of 73 otosclerotic patients Volokhonskaya & Gukovich (1966) noted that generally the sodium concentrations had increased and potassium concentrations had decreased.

Alkaline phosphatase is the most important enzyme associated with ossification. That is why investigators of otosclerosis have always been interested in it. Fowler (1948a) noted that the activity of alkaline phosphatase in serum was within the limits of normal in those otosclerotic patients whose calcium concentrations had not decreased. Riskaer (1949) could not find values different from the normal ones. On the other hand, Maurer (1958) noted that the activity of alkaline phosphatase had decreased. In their well-controlled investigations Soifer et al. (1965) did not observe significant differences in alkaline phosphatase activities in otosclerotic patients and healthy controls.

Activities of other enzymes in the serum of otosclerotic patients have also been examined in order to find a cause for the disease process. Jakabfi et al. (1951) measured cholinesterase activity but did not observe a significant difference compared with normal patients. Timen (1964) noted that the level of cholinesterase activity was elevated after operations for otosclerosis in patients who suffered from vestibular disorders. The corresponding change was not observed in pseudocholinesterase activity.

Otosclerosis often progresses during pregnancy and that is why different questions of hormone metabolism form an interesting group of problems. The connection e.g., between steroids and bone metabolism is a well-known fact. A correlation between otosclerosis and the sex glands was observed as early as in 1937 by Seifert. In addition, he noted that metabolites of parathyroid were increased in otosclerotic patients. Fowler (1948b) emphasized the role of estrogen in otosclerosis. Patroni (1952) again suggested hypofunction of hypophysis, hypogenitalism, hypothyrosis and hyperparathyrosis. Maggio & La Fianza (1952) found decreased secretion of 17-ketosteroids in urine in 47 % of their series. In 25 % the secretion was increased and in 28 % it was normal. Nylén & Nylén (1952) also emphasized the role of the endocrine system in the genesis of otosclerosis. Cassano (1953) stated that secretion of 17-ketosteroids in urine was increased and equally strong in men and women.

Hermann & Maurer (1955) observed, on the other hand, decreased secretion of neutral 17-ketosteroids in otosclerosis. Mau-

In recent years several investigations have also elucidated the appearance of many other enzymes in the otosclerotic focus. Albernaz & Covell (1961) noted that succinic dehydrogenase activity was intensely positive for blood vessels and connective tissue spaces of the otosclerotic focus. Ardouin & Wegmann (1961) observed that there are several disordered enzymes in the focus both with increased and decreased activities. Soifer et al (1969 a) observed decrease in lactate and malate dehydrogenase activities and increase in alkaline phosphatase activity in the otosclerotic stapes

Chevance et al (1963) found cells in the stapes and labyrinthine capsule which were assumed to be able to phosphorylize glucose and form glycogen. Identical cells are seen in the otosclerotic focus but not in healthy bone. Chevance (1964) found sulfhydryl groups in the focus. In his opinion this indicates proteolytic activity Velican & Cinda (1967) revealed in vitro a total disintegration of the matrix of the stapes resembling pathologic lysis of an osteosponginous focus subjected to the action of sialidase and collagenase.

D Microradiography and its application in investigations on otosclerosis

Soft roentgen contact microradiography has made determination of mineral salts in the bone possible (Cosslet et al. 1957 Glimcher 1959) Karlsson et al (1954) were the first investigators to examine the temporal bone by microradiographic methods They prepared 100 to 200 μm thick sections of the malleus and incus and the bony labyrinthine capsule and made microradiographs of the sections Orlandini & Vidoni (1955) examined the effect of chronic otitis media on the auditory ossicles by the same method. Orlandini & Sbernini (1956) employed the method for studying the auditory ossicles of persons at various ages. Frost (1958) developed a method by means of which undecalcified bone sections of the thickness of 3 to 100 μm were made by manual grinding Hallen & Röckert (1960) reported a method by which they made plane-parallel sections. The thickness of these sections could be determined with the accuracy of ± 2 μm Danic et al (1963) examined normal and ostitic auditory ossicles. Ericsson (1965) also developed a method for examining the mineral content of bone The principle of this method is that investigators are able to prepare accurate plane-parallel undecalcified bone sections of which microradiographs are made. The contrast differences of these microradiographs were measured by complicated arrangements in a photometer

Aliprandi (1963) examined otosclerotic foci by microradiographic methods after embedding samples of bone in methyl methacrylate Claree (1964) also determined the degree of mineralization in otosclerotic bone by preparing 40 μm thick undecalcified sections. He used however electron microscopy Garcia Ibañez (1968) examined 46 samples of otosclerotic bone by microradiography by using undecalcified 30 to 50 μm thick sections. He also made traditional histologic preparations of the same samples and divided the foci into four developmental stages. Thus the microradiographs could be grouped in four different stages as well Meurman et al (1968) employed microradiographic methods in examining mineral distribution of otosclerotic foci with different crystalline structures

E. Changes in serum composition associated with otosclerosis

When examining mineralization of the otosclerotic process many investigators have elucidated the possible changes that are reflected in the serum composition Breitmann (1933) noted decrease in the calcium content of serum in some otosclerotic

patients and increase in others. Seifert (1937) observed hypercalcemia and hypophosphatemia and increased secretion of calcium in urine.

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rer (1958) carried out investigations on hormones in patients suffering from otosclerosis and he considered his findings reduced production of androgen Vyslonzil (1961) found even five times as high estrogen levels as normally in male patients decrease of gonadotrophin being always present In as few as 20 per cent the estrogen values were normal and in these cases 17 ketosteroid levels were remarkably low Vyslonzil (1963) found additionally that in male patients the estrogen levels were two to fivefold by the 50th year of age. He thought that the increase was due to elevated activity of Leydig's cells In their series of 73 otosclerotic patients Volokhonskaya & Gukovich (1966) noted depressed level of free 17-oxycorticosteroid secretion both before and after administration of ACTH They regarged it as a disorder of the function of mineralocorticoids in the adrenal cortex. Tarasenkova (1967) noted a direct correlation between the development of clinical manifestations of otosclerosis and the periods of physiologic rise of the estrogen content in the organism

Attempts have been made to elucidate the role of different vitamins in the otosclerotic process by examining the effect of vitamins as a possible treatment and by determining vitamin concentrations in serum (Weber 1932 Koch 1949 Grimaldi 1956 Ferreri 1957) The results of these investigations seem however to be less significant

Riskaer (1949) examined the total protein globulin and albumin in the serum of otosclerotic patients but he did not find any values different from the normal ones Vyslonzil (1954) suggested the alfa and beta globulin levels in serum to be elevated and this he considered to be associated with the hormones of the adrenal

Hlaváček et al (1955) and Hlaváček & Opplt (1957) noted elevated alfa and beta globulin values in otosclerotic patients. Soifer et al (1963) did not find abnormal levels of haptoglobins in serum. In his series of 35 otosclerotic patients Naumann (1964) found decreased albumin values and minor increases in the beta and gamma globulins In their investigation on urinary excretion of amino acids and hexosamine Schoenheyder et al (1966) found no significant variation from control patients.

Senff (1947) found elevated protein levels in cerebrospinal fluid of some otosclerotic patients Schindler et al (1965) analysed the perilymph of 122 otosclerotic patients and noted that it is comparable particularly with capillary serum These investigations were continued by Schindler & Schneider (1966) Chládek & Opplt (1966) noticed a lowered content of alfa and beta globulin fractions in the perilymph of patients with severe otosclerosis Further Chládek & Opplt (1968) established that there was a relationship between the range of the protein spectrum in the perilymph and the activity of otosclerosis.

F Mineral contents of bone

1 Normal bone

Calcium and phosphorus are the most important structural components of hydroxyapatite. Surrounding the crystal there is a halo of adsorbed water molecules and this halo is called the hydration shell. Present in the hydration shell either as solute or adsorbed are such ions as Mg^{++} Sr^{++} $CO_3^{=}$ Na^{+} Cl and citrate and Ca^{++} and PO_4 in some quantities (Frost 1962) Roughly 65 to 70 % of the dry substance of the bone consists of hydroxy apatite crystals (Glimcher 1959) The fatless dry weight of the bone of an adult consists of 26 4 % of calcium 11,3 % of phosphorus 0 18 to 0,6 % of sodium 0,39 of magnesium 0 094 to 0,270 % of fluorine (the amount grows with the age) 0,05 to 0,3 % of potassium 0 011 to 0 017 % of iron 0,0002 to 0 0048 % of copper 0 001 to 0,01 of lead 4 0 % of carbonate and 0 18 % of

chlorine (Documenta Geigy Wissenschaftliche Tabellen, 7 Aufl., 1968)

2. *Normal auditory ossicles and the labyrinthine capsule*

Karlsson et al. (1954) examined mineralization of the auditory ossicles by microradiographic methods and found that the mineral content is low around the haversian canal. In the different parts of the auditory ossicles, the relative mineral contents were remarkably different. Orlandini & Sberuini (1956) noted, also by microradiographic methods, significant variations in the mineral content of the auditory ossicles of persons at different ages. Maurer (1960) examined by chemical analysis the calcium and phosphorus contents of the auditory ossicles and temporal bone of 62 persons. He observed that the mineral contents of the auditory ossicles and labyrinthine capsule were significantly higher than those of the mastoid process and mastoid cortex. The values in men and women did not differ from each other neither did the values between different age groups. Maurer deduced that the organic substance of the auditory ossicles and labyrinthine capsule must differ from that of other bones because the mineral content of the ossicles is higher

Soifer et al. (1970a) examined the mineral contents of the auditory ossicles by neutron activation analysis. In the normal stapes, in an average 29.2±0.8 % was calcium and 11.1 ± 0.2 % phosphorus. Cortical bone samples were taken for control from the posterior wall of the external auditory meatus, and there was in an average 25.8±1.1 % of calcium and 8.2±0.6 % of phosphorus. Evens & Henkin (1969) examined whole mallei and incudes applying the monenergetic photon beam transmission. The stapes was not examined because of its small size. The mineral content of the malleus was significantly higher than that of the incus. No significant differences were noted in either of the bones between the right and left ears. No changes were observed, either in the mineral content of either of the bones in patients between the ages 17 and 60. After 60 however there was possibly decrease in the mineral content.

3. *The auditory ossicles and labyrinthine capsule in otosclerosis*

There are only few among the numerous investigations on otosclerosis which in a reliable way elucidate the possible effects of otosclerosis on the mineral content of the auditory ossicles and labyrinthine capsule. Although several investigators have reported different otosclerotic changes in the auditory ossicles as well (Covell 1940, Nager 1944, Lempert & Wolff 1949) it is a generally accepted view that these changes are secondary involving the stapedial footplate through ingrowth of the otosclerotic focus. Primary otosclerosis in the stapedial footplate is rather rare. On the other hand ossicular changes may develop because of impaired motility of the ossicular chain (Altmann 1965)

Ferreri & Coen (1951) examined the auditory ossicles of healthy persons and otosclerotic patients and found the calcium content to be the same in both groups. Employing electrolytic decalcification, Merlo & Sillingardi (1952) noted that the calcium content in the head of the malleus and incus was 21.6 % and correspondingly 24.8 % lower in otosclerotic than in healthy persons. Covell & Feinmesser (1959) found many different ill-defined changes in their series of 209 ossicles obtained at fenestration operations. Flor & Martinozzi (1960) also found several cases of osteoporosis and bone atrophy in the malleus and incus of otosclerotic patients.

Maurer (1961/62) compared the calcium and phosphorus contents in healthy and otosclerotic persons on one hand in the

ossicles and labyrinthine capsule and on the other hand in the mastoid cortex and mastoid process The mineral contents were at the same level in the mastoid cortex and mastoid process both in healthy and otosclerotic patients On the other hand the calcium and phosphorus contents of the ossicles and labyrinthine capsule were significantly lower in otosclerotic than healthy persons Maurer drew the conclusion that otosclerotic patients have more collagen fibrils in their ossicles and labyrinthine capsule than healthy persons. There were no differences in the mineral contents in the area of lamellar bone (mastoid cortex and mastoid process) on the other hand the differences were distinct in the area of fibrous bone (the auditory ossicles and labyrinthine capsule) between otosclerotic and healthy subjects Clarce (1964) carried out an electron microscopic study on 35 cases of otosclerotic stapes and divided the cases into three groups with the mineral content as a criterion Group 1 represented patients under 28 years of age with a history of progressive deafness for less than 3 years These patients showed some decalcification compared with normal stapes bone. Group 2 consisted of patients younger than 32 years with a history of impaired hearing for less than 3 years These cases were characterized by more marked demineralization. The patients on Group 3 were older than 32 years of age with a history of decreased hearing for more than 3 years They showed increased density indicating some degree of recalcification Röckert et al (1965) examined different parts of the stapes in otosclerotic patients by electron and fluorescence microscopy In mineralization very great individual variation was noted in the head of the stapes *some parts of it were* completely demineralized.

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In their investigation on the role of fluorine in otosclerosis Linck et al (1967) observed that the normal footplate and crura have an equal amount of calcium per unit of weight as normal meatal bone On the other hand even a 100 % otosclerotic foot plate had less calcium than the healthy foot plate According to these investigators, a completely mature otosclerotic bone is unable to reach the high degree of mineralization which is characteristic of the labyrinthine capsule bone. They think however that the calcium content in otosclerotic and *meatal bone is at about the same level.* Garcia Ibañez (1968) suggested that the mineral content of otosclerotic bone is generally lower than that of the healthy stapes Evens and Henkin (1969) applied the monergetic photon beam transmission in examining the malleus and incus of otosclerotic patients The mineral content of the malleus was somewhat lower and that of the incus significantly lower in otosclerotic than in healthy subjects Soifer et al (1970a) examined calcium phosphorus zinc magnesium and sodium concentrations by neutron activation analysis in the stapes and cortical bone in otosclerotic and healthy persons No statistically significant differences were noted Soifer et al (1970b) used the same method *and did not either find* any statistically significant differences in

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The effect of fluoride on inactivation of the otosclerotic process has been investigated with great interest in recent years (Shambaugh & Scott 1964 Shambaugh & Petrovic 1967) In hydroxyapatite the hydroxy ion is replaced by sodium fluoride and thus is fluorapatite formed which is less soluble than normal hydroxyapatite (Arnold 1960) Fluorine once deposited in bone remains there for the life of the individual so that there is a steady increase in the fluorine content of the bones from childhood to old age (Smith et al. 1953) Linck et al. (1967) noted that the normal stapedial footplate and crura have the same amount of fluoride than the normal meatal bone. They also observed that the fluoride content of otosclerotic bone was even three times as high as that of the meatal bone. In their experiments on rats Petrovic & Shambaugh (1968) noted that sodium fluoride promoted bone calcification by decreasing bone resorption and stimulating calcium deposition. Shambaugh (1969) suggested that at its early stage, the otosclerotic focus consists of active areas poor in calcium. It is these areas that sodium fluoride influences.

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IV Material and methods

A. Material

The material consists of bone specimens, which were collected in the Otolaryngological University Clinic Uni ersity of Turku Turku, Finland, in connection with operations for otosclerosis. The specimens were pieces of stapedial footplates including otosclerotic foci. Pieces of stapedial crura were included as well, and the head of the stapes in a few cases The specimens were taken from 250 patients in all of which 70 (174) were women and 30 % (76) men. The age of the patients aried from 7 to 64 years. The mean age in the whole series was 41 7 years the mean age of women being 43.3 years and of men 37.9 years (Table 1) A bone piece of the inner end of the posterior meatal wall was taken from 10 otosclerotic patients.

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Table 1 *Distribution of the material according to age and sex.*

Age	Women	Men
0—10 yrs	1	3
11—20	4	5
21—30	21	13
31—40	45	21
41—50	56	22
51—60	43	11
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In a part of the otosclerotic samples a piece of bone was separated by a knife for further treatment. These pieces were extracted in the Soxhlet apparatus in 80 % ethylendiamine for eight hours and thereafter two more times in the Soxhlet apparatus in distilled water for eight hours. Then the samples were dried at +95°C for 24 hours. Thus it was possible to eliminate most of the organic material from the bone pieces. Some of the samples which were treated with ethylendiamine were, however broken down almost completely during the process. A part of the control material was treated as well in the Soxhlet apparatus in 80 % ethylendiamine.

2. X-ray crystallographic examination

For the examination of the crystalline structure of the bone x-ray diffraction was employed.

III The aim of the study

The aim of the present study is to answer the following questions

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phosphorus determinations the samples were dissolved by nitric acid (Merck, p.a.) and diluted to suitable volume. The calcium and magnesium analyses were carried out by an atom absorption spectrophotometer (Techtron AA-5)

The statistical significances were counted by Student's t test.

The first method was the Debye Scherrer method (Cullity 1956) The diameter of the Debye-Scherrer camera was 114 6 mm. It was used Ni filtered CuK radiation (40 kV and 16 mA) The exposure time was two hours

Preparing of the control samples for the Debye-Scherrer method was done with a file by which certain parts of the ossicles were filed and the powder was packed into Debye Scherrer capillaries with a diameter of 0.2 mm. The ends of the tubes were closed by heating them in a flame. The otosclerotic samples were usually so small that powder samples were not easy to make. That is why bone needles were used which were made with a knife under the operating microscope. Both the glass capillaries and bone needles were fixed in the Debye-Scherrer camera with wax. The wax was not allowed to come under primary radiation. The Debye-Scherrer patterns were measured by a microphotometer (Jarrel Ash Company Mass USA)

For examination of the orientation of bone crystals the Laue transmission method was employed (Cullity 1956) It was used Ni filtered CuK radiation (50 kV and 16 mA) The distance from the specimen to the film was 40 mm and the exposure time varied from one to three hours depending on the thickness of the sample.

Preparing of the control samples for the Laue transmission method was done with a file by which bone plates of the thickness of 200 to 400 μm were made of the ossicles The thickness was defined by a micrometer In the investigation of the otosclerotic material bone sections made for the microradiographic examination were used

3 *Microradiographic examination*

The developmental stage of the otosclerotic focus was examined by microradiographic methods. The bone pieces to be examined were embedded in methylmethacrylate The preparations were sawn about 1 mm thick by a rotating saw The sections were manually ground about 50 to 100 μm thick, and the final grinding was performed by polishing the section with a special glass plate in alcohol The thickness was measured by a micrometer

Soft roentgen contact microradiography was used in the present investigation. It was used Ni filtered CuK radiation (20 kV and 16 mA) Target to film distance was 30 cm. The exposure time was two to six hours. Kodak Maximum Resolution plates were used and developing took place in a Kodak Universal Developer The absorption of x rays was measured by a microscope photometer (Microskop-Photometer MPE, Leitz)

4 *Quantitative mineral analysis*

Neutron activation analysis and atom absorption spectrophotometry were applied. The bone samples were extracted in diethyl ether for 48 hours and thereafter in acetone for 24 hours. Then the samples were dried at +95°C for 72 hours After this, they were cooled for 15 minutes in an exsiccator and weighed (Electromicrobalance EMB-1 Research & Industrial Instruments Company London) The samples and sodium and phosphorus comparative standards were irradiated for two hours in the reactor (Triga Mark II the neutron flux of 2×10^{12} n/cm s) After a delay of 24 hours the γ spectra of the samples and sodium comparative standards were measured and on the basis of the results sodium determinations were carried out The 3 x 3 NaJ (Tl) crystal and 128-channel pulse height analyser (ND-110) were used for γ measurements. After 48 hours the phosphorus contents of the samples were determined on the basis of β measurements The half life measurements showed that the half lives of the activities of bone samples and phosphorus comparative standards were identical After

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For examination of the orientation of bone crystals the Laue transmission method was employed (Cullity 1956) It was used Ni-filtered CuK radiation (50 kV and 16 mA) The distance from the specimen to the film was 40 mm and the exposure time varied from one to three hours depending on the thickness of the sample

Preparing of the control samples for the Laue transmission method was done with a file by which bone plates of the thickness of 200 to 400 μm were made of the ossicles. The thickness was defined by a micrometer In the investigation of the otosclerotic material bone sections made for the microradiographic examination were used.

3 Microradiographic examination

The developmental stage of the otosclerotic focus was examined by microradiographic methods The bone pieces to be examined were embedded in methylmethacrylate. The preparations were sawn about 1 mm thick by a rotating saw The sections were manually ground about 50 to 100 μm thick, and the final grinding was performed by polishing the section with a special glass plate in alcohol The thickness was measured by a micrometer

Soft roentgen contact microradiography was used in the present investigation It was used Ni filtered CuK radiation (20 kV and 16 mA) Target to film distance was 30 cm. The exposure time was two to six hours. Kodak Maximum Resolution plates were used and developing took place in a Kodak Universal Developer The absorption of x rays was measured by a microscope photometer (Mikroskop-Photometer MPE Leitz)

4 Quantitative mineral analysis

Neutron activation analysis and atom absorption spectrophotometry were applied The bone samples were extracted in diethyl ether for 48 hours and thereafter in acetone for 24 hours. Then the samples were dried at +95°C for 72 hours After this they were cooled for 15 minutes in an exsiccator and weighed (Electromicrobalance EMB-1 Research & Industrial Instruments Company London) The samples and sodium and phosphorus comparative standards were irradiated for two hours in the reactor (Triga Mark II the neutron flux of 2×10^{12} n/cm²s) After a delay of 24 hours the γ spectra of the samples and sodium comparative standards were measured and on the basis of the results sodium determinations were carried out The 3 x 3 NaJ (Tl) crystal and 128-channel pulse height analyser (ND-110) were used for γ measurements After 48 hours the phosphorus contents of the samples were determined on the basis of β measurements. The half life measurements showed that the half-lives of the activities of bone samples and phosphorus comparative standards were identical. After

chain, and the direction of the bone plates to their original location was varying. The total of the bone plates was thus 40

The Laue patterns of the bone plates were generally distinct. The bone crystal orientation was best seen in strong 002 reflection. The bone crystals were arranged parallelly with collagen fibrils. In case the c-axes of the bone crystals are approximately perpendicular to the x-ray beam distinct arcing is seen in the 002 reflection as a sign of preferred crystal orientation. In the handle of the malleus the crystals were generally arranged longitudinally with the handle. This was true of the long crus of the incus as well. In the anterior crus of the stapes the crystals were most often parallel with the crus and in the stapedial footplate, they were mostly in line with the edge of the footplate. In the stapedial footplate there was, however great variation in the bone crystal orientation.

2 *Otosclerotic material*

a. The Debye-Scherrer method

All otosclerotic bone samples in the series were examined by the Debye-Scherrer method. Table 2 shows the distribution of the sexes in the material and the mean age of the patients at the time of the operation. The samples examined were collected from 250 patients in all.

After preliminary treatment the bone ieces were examined under an operating microscope. The aim was to find out what *part of the stapes the sample represented*. In most of the samples the original location of the bone piece in the stapes could be found out on the basis of morphological characteristics. In some cases the sample was a whole stapes, from which the wanted part was taken for examination. The general attempt was to choose the part of the bone which morphologically seemed to be most otosclerotic. The samples were divided roughly into three groups A. the sample represented the posterior part of the stapedial footplate including otosclerotic foci B the sample represented the anterior part of the stapedial footplate including otosclerotic foci a distinct separate otosclerotic focus was also included in this group C the sample represented the crus of the stapes (but not the base of the crus) or the head of the stapes. In some cases, the origin of the sample was not quite certain and in these cases the sample was left out. The meaning of this rough division was to localize the bone piece in the otosclerotic stapes.

The total of the investigation material consisted of 20 % (50) of A samples of 75 (187) of B samples and of 5 % (13) of C samples. The low figure of the C samples is explained by the fact that these

able 2 *Distribution of the material in the ...ebye Scherrer examination according to sex ...nd the mean ages of the patients at the time of ...peration.*

Sex		No	Mean age (yrs)
Women	70	(174)	43.3
Men	30	(76)	37.9
Total	100	(250)	41.7

Table 3 *Distribution of the otosclerotic bone material in the Debye-Scherrer examination. Group A. The posterior part of the stapedial footplate. Group B The anterior part ƒ the stapedial footplate or a clear separate otosclerotic focus. Group C The stapedial crura (not the bases of the crura) and the head of the stapes.*

Origin of the sample	%	No.
Group A	20	(50)
Group B	75	(187)
Group C	5	(13)
Total	100	(250)

V Results

A. X ray crystallographic examination

1 Control material

a. The Debye-Scherrer method

For the crystallographic study of normal ossicles the ossicles of five women and five men belonging to the control group were chosen After the preliminary treatment powder samples were made by a file of the following objects 1 head of the malleus 2 manubrium of the malleus 3 body of the incus 4 long crus of the incus 5 head of the stapes 6 stapedial footplate (posterior) 7 stapedial footplate (anterior) 8 external auditory meatus (the inner end of the posterior wall) Ten samples were thus obtained of each object Making of the samples of the malleus and incus was relatively easy but treating the stapes was more difficult It is so brittle that it tended to break while filing it and in addition the stapes is poor in bony material The bone powder of the stapedial footplate was prepared from the base of both crura

Eighty Debye-Scherrer powder patterns were made in all of the normal ossicular chain and posterior wall of the external auditory meatus. Distinct diffraction pat terns were obtained of every sample All diffraction patterns were identical and they completely correspond to those of normal bone i.e. hydroxyapatite presented in th pertinent literature (Carlström 1955)

Most of the diffraction patterns were photometred *The intensity curves perfectly* corresponded to the curves of the diffraction patterns of normal bone.

In order to eliminate the disturbing back ground scattering caused by the organic component of the bone as carefully as possible a part of the normal ossicular chain was treated in the Soxhlet apparatus in 80 % ethylendiamine. Of the bony material thus obtained two powder samples were made of each of the eight objects mentioned above. Thus the diffraction lines became nar rower and sharper so that it was possible to observe even minor changes in the patterns. Even these Debye-Scherrer patterns were photometred Neither in these technically better diffraction patterns were any dif ferences from the pattern of normal hydr oxyapatite.

On the basis of the examinations reported above it is suggested that there are no crystalline structures in the normal ossicular chain and temporal bone (external meatus) that examined by the Debye-Scherrer meth od would differ from the crystalline struc ture of normal human bone

b The Laue transmission method

For the study of crystal orientation in normal auditory ossicles ossicles of five female and five male controls were chosen Thin bone plates were made of the cleaned ossicles by a file The thickness of the bone plates was generally 200 μm but some of the plates were as thick as 400 μm In these cases the exposure time was correspondingly made longer Bone plates were prepared *from the following objects 1* manubrium of the malleus 2. long crus of the incus 3 anterior crus of the stapes 4 stapedial footplate (anterior) When making the preparations the direction of the prepara tion plate in relation to its original location was known Thus it was possible to draw conclusions of crystal orientation in the original part of the ossicl T n pr parations were made of each part of the ossicular

chain, and the direction of the bone plates to their original location was varying. The total of the bone plates was thus 40.

The Laue patterns of the bone plates were generally distinct. The bone crystal orientation was best seen in strong 00'2 reflection. *The bone crystals were arranged parallelly with collagen fibrils.* In case the c-axes of the bone crystals are approximately perpendicular to the x-ray beam, distinct arcing is seen in the 00'2 reflection as a sign of preferred crystal orientation. In the handle of the malleus the crystals were generally arranged longitudinally with the handle. *This was true of the long crus of the incus* as well. In the anterior crus of the stapes, the crystals were most often parallel with the crus, and in the stapedial footplate they were mostly in line with the edge of the footplate. In the stapedial footplate there was, however great variation in the bone *crystal orientation.*

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The total of the investigation material consisted of 20 % (50) of A samples, of 75 % (187) of B samples and of 5 % (13) of *C samples. The low figure of the C* samples is explained by the fact that these

Table 2. *Distribution of the material in the Debye-Scherrer examination according to sex and the mean ages of the patients at the time of operation.*

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Origin of the sample	%	No.
Group A	20	(50)
Group B	75	(187)
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V Results

A. X ray crystallographic examination

1 Control material

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a

b

c

Fig. 2. Deby -Scherrer diffraction patterns of otosclerotic bone. Patterns of normal hydroxy apatite (a—c)

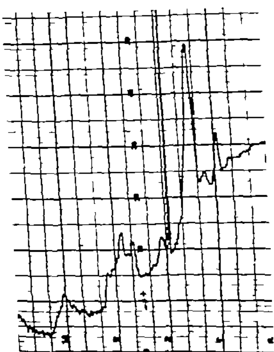


Fig. 3. Intensity curv of normal hydroxyapatite of the diffraction pattern in Fig. 2.

mean age at the time of the operation was 42.4 years in this group. Figure 7 presents some diffraction patterns interpreted to represent the amorphous structure. Diffraction lines typical of hydroxyapatite are lacking for the most part. A photometer curve of the Debye-Scherrer pattern in Figure 7 (c) is seen in Figure 8. The figure presents a typical x-ray intensity curve of amorphous substance (Cullity 1956).

The total figure of samples with a crystalline structure deviating from the structure of normal hydroxyapatite was thus 25 % (62) in all. Of these, 74 % (46) were taken from women and 26 % (16) from men. The mean age of all abnormal cases was 41.6 years.

When the obtained crystalline structure types of the otosclerotic bone are analysed according to the *original site of the sample* in the stapes it is noted that 19 % (36) of the samples with normal crystalline *structure* belonged to Group A (the poste-

Table 4 *The results of the Debye-Scherrer examination*

Samples from	Normal crystalline structure %	No	Abnormal crystalline structure %	No.	Amorphous structure %	No.	Abnormal plus Amorphous %	No.
Women	68	(128)	83	(15)	70	(31)	74	(46)
Men	32	(60)	17	(3)	30	(13)	26	(16)
Total	100	(188)	100	(18)	100	(44)	100	(62)

samples were examined only in case the stapedial footplate or a distinct otosclerotic focus was not available (Table 3) If the material was sufficient several Debye-Scherrer patterns were made of the stapes of the same patient alternating somewhat however the part from which the sample was taken Generally repeated photography gave a similar pattern but in some cases deviations from earlier photographs were noted. In these cases the deviating Debye-Scherrer pattern only was included For analysing the results the diffraction patterns of the otosclerotic material were compared with the diffraction pattern of normal hydroxyapatite. All diffraction patterns that were or were suspected to be different from normal were measured by the photometer Thereafter the Debye Scherrer patterns were divided into three main groups 1 Normal crystalline structure the Debye-Scherrer pattern is identical with the pattern of normal hydroxyapatite 2 Abnormal crystalline structure the Debye-Scherrer pattern deviates more or less from the pattern of normal hydroxyapatite The abnormality may e.g be deviation of some of the diffraction lines from its normal location strengthening or weakening of the relative intensity of the diffraction line or appearance of a new additional diffraction line 3 Amorphous structure no well-defined intensity maxima are noted in the Debye-Scherrer pattern but only diffuse and broad lines Dividing the results into three groups only is very rough because the limits between the different groups are at least partly diffuse. The division was however done in order to make the analysis of the results easier

Table 4 shows the distribution of the otosclerotic samples into different structural types on the basis of the Debye-Scherrer examination Seventy five per cent (188) of the 250 samples examined had a diffraction pattern that was considered normal. Of these samples 68 % (128) were taken from women and 32 % (60) from men The mean age at the time of the operation was 41 7 years in this group Figure 2 shows three Debye-Scherrer diffraction patterns regarded as normal and in Figure 3 the corresponding normal photometer curve is seen A diffraction pattern interpreted abnormal was found in 7 % (18) of the samples Of these samples 83 % (15) were taken from women and 17 % (3) from men In this group the mean age at the time of the operation was 39 4 years Figure 4 shows some Debye Scherrer diffraction patterns which have been considered to differ from the pattern of normal hydroxyapatite Attention must be paid to the fact that there are diffraction patterns of several different types In Figure 5 an intensity curve of the Debye-Scherrer pattern of Figure 4 (d) is seen and correspondingly Figure 6 presents the photometer curve of the Debye-Scherrer pattern seen in Figure 4 (g) A structure considered amorphous was noted in 18 % (44) of the samples of these 70 (31) wer from women and 30 % (13) from men Th

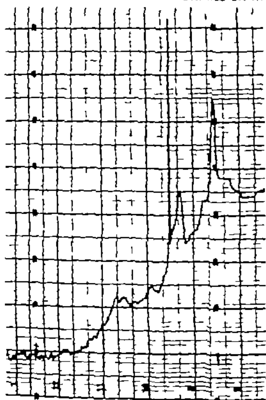


Fig. 5. Intensity curve of the Debye-Scherrer diffraction pattern in Fig. 4 (d)

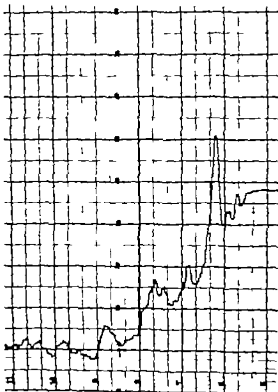


Fig. 6. Intensity curve of the Debye-Scherrer diffraction pattern in Fig. 4 (g)

rior part of the stapes) 74 % (139) to Group B (the anterior part of the stapes) and 7 % (13) to Group C (the crus or head of the stapes). Group A consisted of 39 % (7), Group B 61 % (11) and Group C none of the samples with the abnormal crystalline structure. Of samples with an amorphous structure 16 % (7) belonged to Group A, 84 % (37) to Group B and none to Group C. Thus of the samples with a structure different from the crystalline structure of normal hydroxyapatite (abnormal crystalline structure plus amorphous structure) 23 % (14) belonged to Group A, 77 % (48) to

Table 5. *Distribution of the results of the Debye-Scherrer examination according to the origin of the samples. Groups A, B and C see Table 3.*

Origin	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure		Abnormal plus Amorphous	
	%	No	%	No	%	No.	%	No.
Group A	19	(36)	39	(7)	16	(7)	23	(14)
Group B	74	(139)	61	(11)	84	(37)	77	(48)
Group C	7	(13)	—	(—)	—	(—)	—	(—)
Total	100	(188)	100	(18)	100	(44)	100	(62)

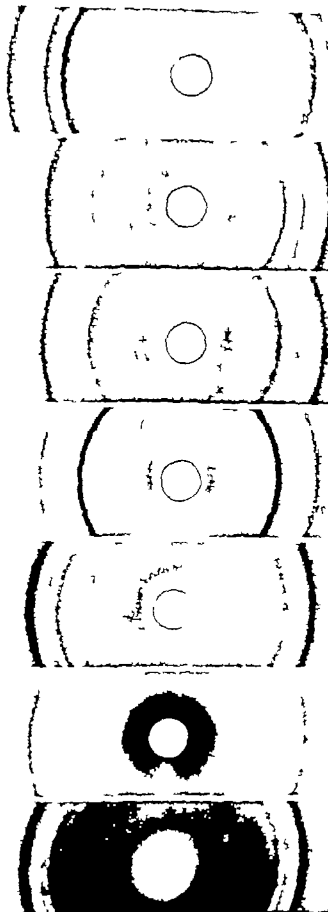


Fig 4 Debye-Scherrer diffraction patterns of otosclerotic bone different from the pattern o normal hydroxyapatite (a—g)

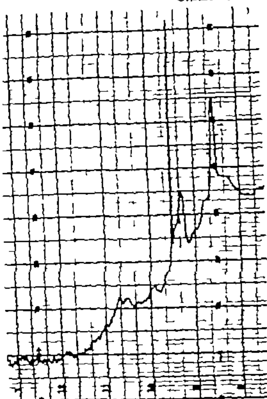


Fig. 5. Intensity curve of the Debye-Scherrer diffraction pattern in Fig. 4 (d)

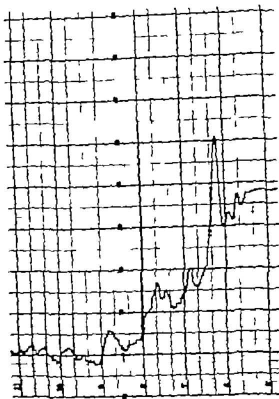


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Total	100	(188)	100	(18)	100	(44)	100	(62)

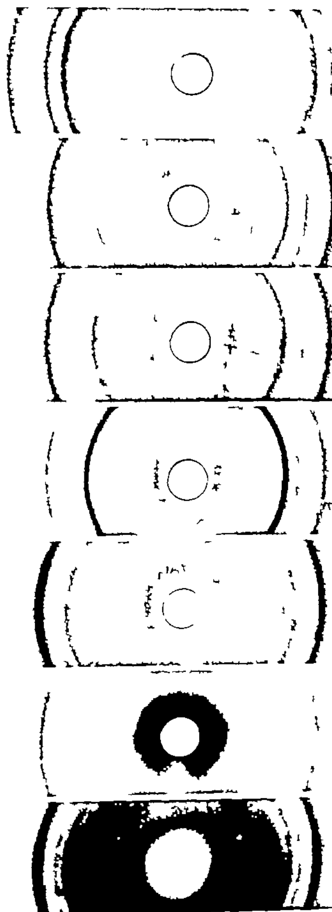


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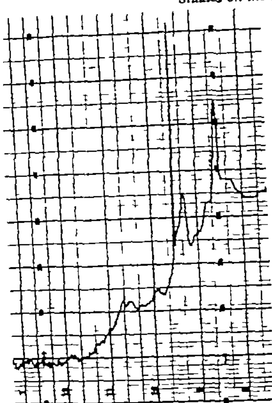


Fig. 5 Intensity curve of the Debye-Scherrer diffraction pattern in Fig. 4 (d)

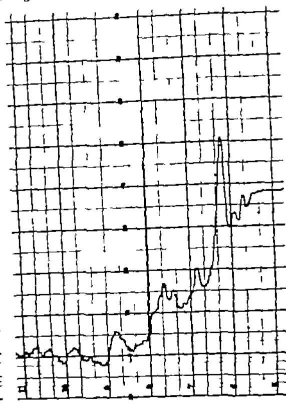


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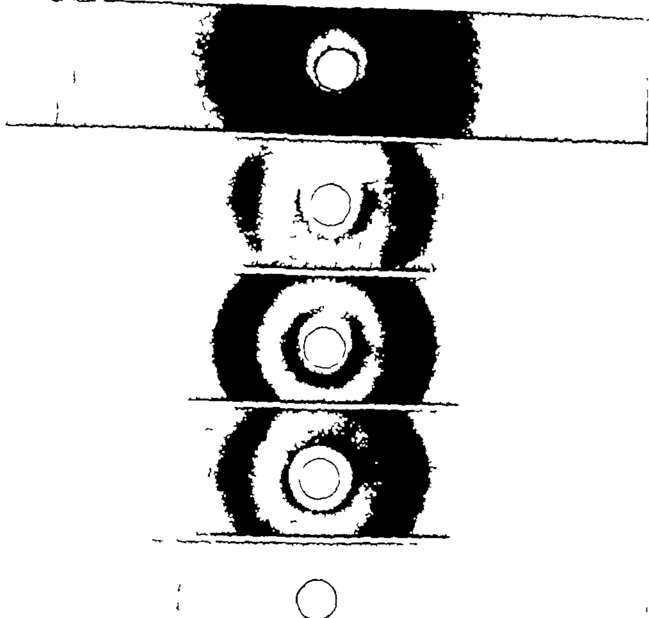


Fig 7 Debye-Scherrer diffraction patterns of otosclerotic bone. The patterns (a—e) indicate *amorphous structure*.

Group B and none to Group C Examined by the Debye-Scherrer method the crystal line structure of the bone was normal in all cases in the crura and head of the stapes (Table 5)

Since it is generally accepted that otosclerotic changes do not occur in upper parts of the crura or in the head of the stapes (≈Group C) samples belonging to Group C can be left out Then the percentages of the crystalline structure different from normal hydroxyapatite become higher Table 6 shows that samples in the groups A and B were 237 in all. Of these 73 % (175) were considered to represent the structure of normal hydroxyapatite The abnormal crystalline structure was noted in 8 % (18) of the samples, and an amorphous structure in 19 % (44) of the samples. Twenty seven per cent (62) of these samples belonging to

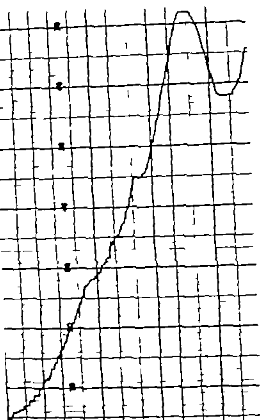


Fig. 8. Intensity curve of the Debye-Scherrer diffraction pattern in Fig. 7 (c). Typical curve of amorphous substance.

groups A and B were shown to have a structural deviation from normal hydroxyapatite.

Table 6. Distribution of the result of the Debye-Scherrer examination. Only the stapedial footplate is included.

Crystalline structure	%	No.
Normal	73	(175)
Abnormal	8	(18)
Amorphous	19	(44)
Abnormal plus Amorphous	27	(62)
Total	100	(237)

Table 7. Distribution of the results of the Debye-Scherrer examination. Sixty samples extracted by ethylendiamine were included.

Crystalline structure	%	No.
Normal	85	(51)
Abnormal	12	(7)
Amorphous	3	(2)
Abnormal plus Amorphous	15	(9)
Total	100	(60)

In order to make the grouping of the samples more reliable a part of the samples were extracted in ethylendiamine in the Soxhlet apparatus after the native photographing described above. These samples were 26 % (66) of the total material, and of these 66 65 % (43) were taken from women and 35 % (23) from men. During the treatment six samples were broken down so that the Debye-Scherrer patterns were obtained of 60 samples. A normal diffraction pattern was noted in 85 % (51) of the samples. The abnormal crystalline structure was found in 12 % (7) of the samples and amorphous structure in 3 % (2). Structures deviating from normal hydroxyapatite were thus in 15 % (9) in all (Table 7). It is probable that most of the samples that were broken down during the ethylendiamine extraction belonged to amorphous cases. Most of the diffraction patterns obtained from samples treated with ethylendiamine were identical with earlier results. The technically better Debye-Scherrer patterns thus obtained were in three cases considered abnormal instead of regarding them as normal on the basis of native photographing.

A piece of bone from the medial part of the posterior meatal wall of 10 otosclerotic patients were also examined by making Debye-Scherrer powder patterns of them. All diffraction patterns thus obtained were

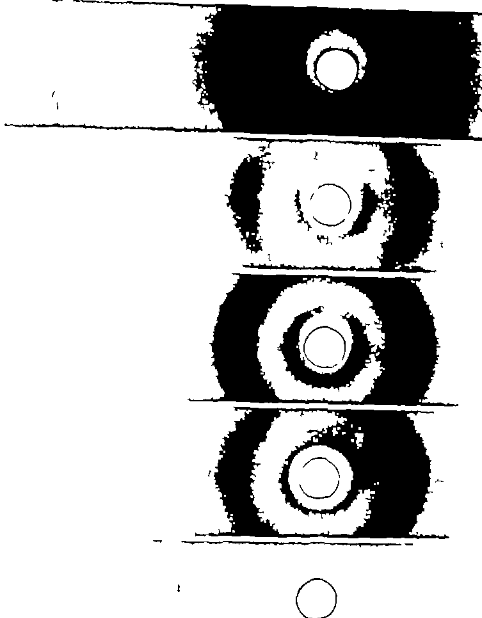


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Table 9 *Results of the Laue examination.*

Samples from	Preferred orientation		No preferred orientation		Indistinct Laue pattern (amorphous)	
	%	No.	%	No.	%	No.
Women	67	(8)	76	(26)	75	(3)
Men	33	(4)	24	(8)	25	(1)
Total	100	(12)	100	(34)	100	(4)

Table 9 shows the results of the crystal orientation investigation. Out of the 50 examined samples in 24 % (12) a more or less clearly preferential bone crystal orientation was seen. Sixty-seven per cent (8) of these were taken from women and 33 % (4) of men. Figure 9 presents a Laue pattern, in which distinct intensity maxima and minima are seen in strong 00 2 reflection as a sign of preferred bone crystal orientation No orientation, according to the Laue pattern was seen in 68 % (34) of the samples of which 76 % (26) were from women and 24 % (8) from men. In the Laue pattern in Figure 10 the arcing phenomenon is not seen, and this means that the bone crystals in this sample are not clearly oriented. Four samples did not give a clear diffraction photograph at all, but only a typical pattern of the amorphous structure.

Table 10. *Distribution of the results of the Laue examination. The crystalline structures are based on the Debye-Scherrer examination.*

Bone crystal orientation	Normal crystalline structure		Abnormal crystalline structure	
	%	No.	%	No.
Preferred orientation	25	(10)	20	(2)
No preferred orientation	65	(26)	80	(8)
Indistinct Laue pattern (amorphous)	10	(4)	—	(—)
Total	100	(40)	100	(10)

The distribution of bone crystal orientation into normal or abnormal cases on the basis of the Debye-Scherrer examination is seen in Table 10 In the samples with normal crystalline structure in 25 % (10) orientation was noted, in 65 % (26) there was no preferred orientation and 10 % (4) gave a diffraction pattern typical of the amorphous structure. On the other hand in 80 % (8) of the samples with the abnormal crystalline structure, no perferred orientation was observed.

B. Microradiographic examination

Fifty bone pieces were arbitrarily selected from the otosclerotic cases for the microradiographic examination. Yet, all samples belonged to Group B because the samples were chosen to be as otosclerotic as possible for this examination. Table 11 shows the composition of the material. Another principle for selection was to include 30 bone pieces with normal crystalline structure 10 with abnormal crystalline structure and 10 with amorphous structure. Of the first mentioned 77 % (23) cases were from women and 23 % (7) from men, in the second group 80 % (8) were from women and 20 % (2) from men and in the last mentioned group 0 % (7) were from women and 30 % (3) from men. Thus, in the microradiographic examination 76 % (38) of the samples were from women and 24 % (12) from men. The mean age of the whole group was 41 7 years at the time of the operation in women 42.9 and in men 38.1 years.

The original aim of the microradiographic examination was to determine the quantitative mineral contents in otosclerotic bone samples by the soft roentgen contact microradiographic technique. For this reason, a reference system made of aluminium foils was placed next to the bone sections during

identical with the diffraction pattern of hydroxyapatite.

b The Laue transmission method

In the crystal orientation examination of otosclerotic samples methylmethacrylate sections prepared for microradiographic examination were used. These sections could be fixed in the Laue camera without special arrangements. Now it was not, however possible to know in which direction the thin section was originally cut from the bone. That is why the present author could only find out whether the bone crystals were oriented at all The direction of the orientation could thus not be elucidated.

Samples for the crystal orientation study were chosen partly arbitrarily from the original samples All selected bone pieces belonged to Group B in other words they represented the anterior part of the otosclerotic footplate or a clear separate otosclerotic focus Forty samples with the normal crystalline structure were taken Out of these samples 73 % (29) were from women and 27 % (11) from men. In addition 10 samples with the abnormal crystalline structure were included and 80 % (8) of these were from women and 20 % (2) from men The number of bone samples thus totalled up to 50 Samples with the amorphous structure according to the Debye-Scherrer investigation were all excluded (Table 8)

Table 8. *Distribution of the bone material in the Laue examination. The crystalline structures are based on the Debye-Scherrer examination.*

Samples from	Normal crystalline structure		Abnormal crystalline structure	
	%	No.	%	No.
Women	73	(29)	80	(8)
Men	27	(11)	20	(2)
Total	100	(40)	100	(10)

The Laue patterns were divided into two groups 1 bone crystals with more or less preferential orientation and 2 bone crystals with no preferred orientation. If the arcing phenomenon was not noted in the 002 reflection the samples were counted to the second group

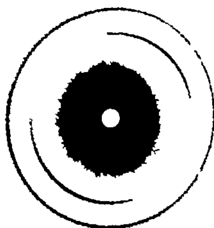


Fig 9 Laue transmission pattern of otosclerotic bone. Clear arcing in 002 reflection as a sign of preferred bone crystal orientation.

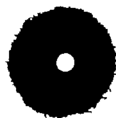


Fig 10 Laue transmission pattern of otosclerotic bone. No clear arcing in 002 reflection showing lack of preferred bone crystal orientation.

Table 9 *Results of the Laue examination*

Samples from	Preferred orientation		No preferred orientation		Indistinct Laue pattern (amorphous)	
	%	No.	%	No.	%	No.
Women	67	(8)	76	(26)	75	(3)
Men	33	(4)	24	(8)	25	(1)
Total	100	(12)	100	(34)	100	(4)

Table 9 shows the results of the crystal orientation investigation. Out of the 50 examined samples in 24 % (12) a more or less clearly preferential bone crystal orientation was seen. Sixty seven per cent (8) of these were taken from women and 33 % (4) of men. Figure 9 presents a Laue pattern in which distinct intensity maxima and minima are seen in strong 00 2 reflection as a sign of preferred bone crystal orientation. No orientation according to the Laue pattern, was seen in 68 % (34) of the samples, of which 76 % (26) were from women and 24 (8) from men. In the Laue pattern in Figure 10 the arcing phenomenon is not seen and this means that the bone crystals in this sample are not clearly oriented. Four samples did not give a clear diffraction

Table 10 *Distribution of the results of the Laue examination. The crystalline structures are based on the Debye-Scherrer examination*

Bone crystal orientation	Normal crystalline tructure		Abnormal crystalline structure	
	%	No	%	No
Preferred orientation	25	(10)	20	(2)
No preferred orientation	65	(26)	80	(8)
Indistinct Laue pattern (amorphous)	10	(4)	—	(—)
Total	100	(40)	100	(10)

photograph at all but only a typical pattern of the amorphous structure.

The distribution of bone crystal orientation into normal or abnormal cases on the basis of the Debye-Scherrer examination is seen in Table 10 In the samples with normal crystalline structure, in 25 % (10) orientation was noted in 65 % (26) there was no preferred orientation and 10 (4) gave a diffraction pattern typical of the amorphous structure. On the other hand, in 80 % (8) of the samples with the abnormal crystalline structure no perferred orientation was observed.

B. Microradiographic examination

Fifty bone pieces were arbitrarily selected from the otosclerotic cases for the microradiographic examination. Yet, all samples belonged to Group B because the samples were chosen to be as otosclerotic as possible for this examination. Table 11 shows the composition of the material. Another principle for selection was to include 30 bone pieces with normal crystalline structure, 10 with abnormal crystalline structure and 10 with amorphous structure. Of the first mentioned, 77 % (23) cases were from women and 23 % (7) from men, in the second group 80 % (8) were from women and 20 % (2) from men and in the last mentioned group 70 % (7) were from women and 30 % (3) from men. Thus, in the microradiographic examination, 76 % (38) of the samples were from women and 24 (12) from men. The mean age of the whole group was 41.7 years at the time of the operation, in women 42.9 and in men 38.1 years.

The original aim of the microradiographic examination was to determine the quantitative mineral contents in otosclerotic bone samples by the soft roentgen contact microradiographic technique. For this reason, a reference system made of aluminium foils was placed next to the bone sections during

Fig 11 Microradiograph representing the fourth developmental stage of otosclerotic bone. The marrow spaces are greatly enlarged. Curving bone trabeculae join each other and there are a great number of osteocytic lacunae irregular in shape. The typical honeycomb-like structure is seen in some places. In the Debye-Scherrer examination, this sample gave a diffraction pattern typical of amorphous structure.

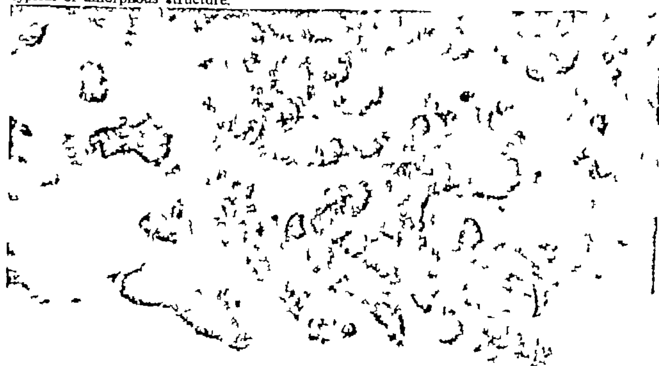


Fig. 12 Microradiograph representing the third developmental stage of otosclerotic bone. Osteocytic lacunae are fewer and the trabeculae thicker The marrow spaces are more scarce than in the fourth stage focus. In the Debye-Scherrer examination a diffraction pattern showing abnormal crystalline structure was obtained of this sample

Fig 13 Microradiograph, which represents the second developmental tage of otosclerotic bone. The marrow spaces are more scarce than t the third stage. The trabeculae are thick, and the osteocytic lacunae are fewer and more regular The crystalline structure examination revealed abnormal structure in this sample.

I g 14 Microradiograph representing the otosclerotic focus at the first developmental stage. Thi tage is the most matu e. A distinct tendency to lamellar more regular structure is noted. The tructure as whole is more compact. The number of osteocytic lacunae is only somewhat greater than normally I the Debye Scherrer examination, this sample gave a diffraction pattern of normal rystalline structure

Fig 11 Microradiograph representing the fourth developmental stage of otosclerotic bone. The marrow spaces are greatly enlarged. Curving bone trabeculae join each other and there are a great number of osteocytic lacunae irregular in shape. The typical honeycomb-like structure is seen in some places In the Debye-Scherrer examination this sample gave a diffraction pattern typical of amorphous structure.

Fig 12 Microradiograph representing the third developmental stage of otosclerotic bone. Osteocytic lacunae are fewer and the trabeculae thicker The marrow spaces are more scarce than in the fourth stage focus. In the Debye-Scherrer examination, a diffraction pattern showing abnormal crystalline structure was obtained of this sample.



Fig 13. Microradiograph, which represents the second developmental stage of otosclerotic bone. The marrow spaces are more scarce than at the third tage. The trabeculae are thick, and the osteocytic lacunae are fewer and more regular The cry talline structure examination revealed abnormal structure in this sample.

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Table 11 *Distribution of the bone material in the microradiographic examination according to the crystalline structure.*

Samples from	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure	
	%	No	%	No	%	No
Women	77	(23)	80	(8)	70	(7)
Men	23	(7)	20	(2)	30	(3)
Total	100	(30)	100	(10)	100	(10)

the exposure The thickness of this aluminium stepwedge was 15 30 45 150 μm Thereafter the microradiographs were measured by photometry and the mineral contents of the bone pieces were thus attempted to be determined quantitatively A great margin for errors was however revealed and the author had to give up any conclusions. Still it seemed evident that bone samples which had amorphous structure according to the Debye-Scherrer method contained less minerals than the samples with normal or abnormal crystalline structure

On the basis of the microradiographs obtained the otosclerotic samples were divided into different developmental stages Four different stages were grouped according to Henner et al (1960) and Garcia Ibañez (1968) The meaning of the grouping was to find out at what developmental stage of otosclerosis abnormal crystalline structures appear It must be kept in mind however that the otosclerotic samples in the present investigation were small in size, which caused difficulties.

Table 12. *Results of the microradiographic examination. The developmental stages of otosclerotic bone according to the crystalline structure.*

Developmental stage	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure	
	%	No	%	No	%	No
IV	30	(9)	30	(3)	100	(10)
III	30	(9)	40	(4)	—	(—)
II	17	(5)	20	(2)	—	(—)
I	23	(7)	10	(1)	—	(—)
Total	100	(30)	100	(10)	100	(10)

Table 12 shows the results of the microradiographic grouping The results reveal that the samples with amorphous structure represent the youngest most active otosclerotic foci. On the other hand, abnormal crystalline structures may apparently occur at all developmental stages of the otosclerotic process. Of the total number of the samples 44 % (22) belonged to the fourth 26 % (13) to the third 14 % (7) to the second and 16 % (8) to the first developmental stage

C. Quantitative mineral analysis

1 Control material

After preliminary treatment a small bone piece was taken from the anterior part of the footplate of the control stapedes. The anterior part was chosen for the reason that a control material as good as possible was wanted for the otosclerotic material which mostly contained samples of the anterior part of the stapedial footplate. The number of the control samples was 20 The samples were dried to constant weight and weighed whereafter sodium and phosphorus contents were determined quantitatively by neutron activation analysis and calcium and magnesium contents by atom absorption spectrophotometry

Table 13 shows the constant weight and calcium phosphorus sodium and magnesium contents of each sample in mg/100 mg bone. The table reveals that the average content of calcium in the normal stapedial footplate is 30.28 of phosphorus 15 96 of sodium 0 43 and of magnesium 0.34

Table 13. *Ca, P Na and Mg contents of normal stapedial footplate (the anterior part) in mg/100 mg bone.*

Sample No.	Weight (mg)	Ca	P	Na	Mg
1	0.411	33.6	17.8	0.36	0.35
2	0.615	26.7	14.2	0.35	0.29
3	0.664	26.0	13.5	0.52	0.43
4	0.342	37.2	17.6	0.35	0.34
5	0.490	27.6	13.4	0.53	0.41
6	0.919	36.6	16.8	0.35	0.48
7	0.468	26.7	13.8	0.61	0.34
8	0.272	27.6	14.9	0.53	0.31
9	0.266	29.3	17.0	0.59	0.40
10	0.290	37.2	19.0	0.29	0.29
11	0.542	26.8	16.6	0.29	0.28
12	0.252	28.2	15.2	0.94	0.41
13	0.687	35.3	19.7	0.31	0.21
14	1.740	28.6	11.6	0.50	0.38
15	0.655	35.9	19.2	0.25	0.20
16	0.529	35.9	18.3	0.30	0.23
17	0.294	27.9	15.1	0.31	0.20
18	0.443	25.2	15.6	0.11	0.38
19	0.689	25.8	14.0	0.58	0.49
20	0.259	27.4	15.9	0.52	0.35
Mean $(\bar{x})$	0.544	30.28	15.96	0.43	0.34

2. *Otosclerotic material*

Forty samples were selected partly arbitrarily from the otosclerotic bone samples for quantitative mineral analysis. All samples belonged to Group B in other words they represented the anterior part of the otosclerotic stapedial footplate or a clear separate otosclerotic focus. The aim was to obtain as otosclerotic samples as possible for this examination The composition of the material is given in Table 14 An additional principle was to include 20 samples with normal crystalline structure 10 samples with abnormal crystalline structure and 10 samples with amorphous structure. Of the first mentioned 65 % (13) were from women and 35 (7) from men. Of the second group 50 (5) were from women and 50 (5) from men, and in the last group, 40 (4) from women and 60 (6) from men. Sodium and phosphorus contents were determined quantitatively by neutron activation analysis and calcium and magnesium contents by atom absorption spectrophotometry

Table 15 shows the constant weights and calcium, phosphorus, sodium and magnesi-

Table 14. *Distribution of the otosclerotic bone material in quantitative mineral analysis. The crystalline structures are based on the Debye-Scherrer examination.*

Samples from	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure	
	%	No.	%	No.	%	No.
Women	65	(13)	50	(5)	40	(4)
Men	35	(7)	50	(5)	60	(6)
Total	100	(20)	100	(10)	100	(10)

Table 15. *Ca, P Na and Mg contents of otosclerotic stapedial footplate in mg/100 mg bone. All samples represented the diffraction pattern of normal hydroxyapatite.*

Sample No.	Weight (mg)	Ca	P	Na	Mg
1	0.686	25.1	14.1	0.30	0.22
2	0.192	24.5	14.2	0.22	0.13
3	0.250	25.4	14.3	0.52	0.15
4	0.411	33.9	17.2	0.36	0.35
5	0.545	25.7	15.4	0.60	0.30
6	0.749	26.4	13.5	0.47	0.37
7	1.240	28.7	11.5	0.54	0.37
8	0.217	23.0	12.9	0.31	0.09
9	0.744	23.5	15.7	0.56	0.29
10	1.550	25.8	13.0	0.50	0.29
11	0.664	26.6	13.8	0.54	0.38
12	0.782	24.9	13.8	0.21	0.35
13	0.280	36.6	18.0	0.28	0.38
14	0.606	23.1	13.0	0.45	0.27
15	0.791	22.3	15.2	0.13	0.06
16	0.438	21.1	11.4	0.48	0.34
17	0.259	27.1	15.6	0.50	0.33
18	0.642	22.3	11.7	0.54	0.31
19	0.490	25.8	10.8	0.39	0.12
20	0.890	23.2	10.2	0.37	0.12
Mean $(\bar{x})$	0.621	25.85	13.87	0.41	0.26

Table 11 *Distribution of the bone material in the microradiographic examination according to the crystalline structure.*

Samples from	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure	
	%	No	%	No	%	No
Women	77	(23)	80	(8)	70	(7)
Men	23	(7)	20	(2)	30	(3)
Total	100	(30)	100	(10)	100	(10)

the exposure The thickness of this aluminium stepwedge was 15 30 45 150 µm. Thereafter the microradiographs were measured by photometry and the mineral contents of the bone pieces were thus attempted to be determined quantitatively A great margin for errors was however revealed, and the author had to give up any conclusions Still it seemed evident that bone samples which had amorphous structure according to the Debye-Scherrer method contained less minerals than the samples with normal or abnormal crystalline structure.

On the basis of the microradiographs obtained the otosclerotic samples were divided into different developmental stages Four different stages were grouped according to Henner et al. (1960) and Garcia Ibañez (1968) The meaning of the grouping was to find out at what developmental stage of otosclerosis abnormal crystalline structures appear It must be kept in mind however that the otosclerotic samples in the present investigation were small in size which caused difficulties

Table 12 shows the results of the microradiographic grouping. The results reveal that the samples with amorphous structure represent the youngest, most active otosclerotic foci. On the other hand, abnormal crystalline structures may apparently occur at all developmental stages of the otosclerotic process. Of the total number of the samples 44 % (22) belonged to the fourth 26 % (13) to the third 14 % (7) to the second and 16 % (8) to the first developmental stage.

Table 12. *Results of the microradiographic examination. The developmental stages of otosclerotic bone according to the crystalline structure.*

Developmental stage	Normal crystalline structure		Abnormal crystalline structure		Amorphous structure	
	%	No	%	No	%	No
IV	30	(9)	30	(3)	100	(10)
III	30	(9)	40	(4)	—	(—)
II	17	(5)	20	(2)	—	(—)
I	23	(7)	10	(1)	—	(—)
Total	100	(30)	100	(10)	100	(10)

C. Quantitative mineral analysis

1 Control material

After preliminary treatment a small bone piece was taken from the anterior part of the footplate of the control stapedes. The anterior part was chosen for the reason that a control material as good as possible was wanted for the otosclerotic material which mostly contained samples of the anterior part of the stapedial footplate The number of the control samples was 20 The samples were dried to constant weight and weighed, whereafter sodium and phosphorus contents were determined quantitatively by neutron activation analysis and calcium and magnesium contents by atom absorption spectrophotometry

Table 13 shows the constant weight and calcium phosphorus sodium and magnesium contents of each sample in mg/100 mg bone. The table reveals that the average content of calcium in the normal stapedial footplate is 30.28 of phosphorus 15 96 of sodium 0 43 and of magnesium 0.34

Table 19. *Comparison between the Ca, P, Na and Mg contents of normal stapedial footplate and otosclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone). The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal stapes	30.28	18.03	2.82	0.01	28
	Abnormal otos. bone	24.70	37.48			
P	Normal stapes	15.96	4.64	1.72	0.1	28
	Abnormal otos. bone	14.27	8.75			
Na	Normal stapes	0.43	0.03	1.98	—	28
	Abnormal otos. bone	0.36	0.02			
Mg	Normal stapes	0.34	0.01	2.75	0.01	28
	Abnormal otos. bone	0.23	0.01			

Table 20. *Comparison between the Ca, P, Na and Mg contents of normal stapedial footplate and otosclerotic bone with amorphous structure (=amorphous otosclerotic bone). The mean contents ($\bar{x}$) in mg/100 mg bone*

		$\bar{x}$	s^2	t	p	df
Ca	Normal stapes	30.28	18.03	6.96	0.001	28
	Amorphous otos. bone	18.24	19.55			
P	Normal stapes	15.96	4.64	6.18	0.001	28
	Amorphous otos. bone	10.51	7.34			
Na	Normal stapes	0.43	0.03	1.29	—	28
	Amorphous otos. bone	0.35	0.03			
Mg	Normal stapes	0.34	0.01	4.00	0.001	28
	Amorphous otos. bone	0.18	0.01			

Table 21. *Comparison between the Ca, P, Na and Mg contents of otosclerotic bone with normal crystalline structure (=normal otosclerotic bone) and of sclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone). The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal otos. bone	25.85	13.25	0.62	—	28
	Abnormal otos. bone	24.70	37.48			
P	Normal otos. bone	13.87	3.74	0.43	—	28
	Abnormal otos. bone	14.27	8.75			
Na	Normal otos. bone	0.41	0.02	0.89	—	28
	Abnormal otos. bone	0.36	0.02			
Mg	Normal otos. bone	0.26	0.01	0.75	—	28
	Abnormal otos. bone	0.23	0.01			

ingly the phosphorus content of this kind of otosclerotic bone (13.87) is significantly lower than that of normal stapedial bone (15.96) ($p<0.01$). Between the sodium contents of normal stapedial bone (0.43) and otosclerotic bone with normal crystalline structure (0.41) no statistically significant difference was noted. On the other hand, the magnesium content of otosclerotic bone (0.26) is nearly significantly lower than that of normal stapedial bone (0.34) ($p<0.05$).

um contents of otosclerotic samples with normal crystalline structure in mg/100 mg bone The average content of calcium in this group is 25 85 of phosphorus 13.87 of sodium 0 41 and of magnesium 0.26

Table 16 shows the constant weights and calcium phosphorus sodium and magnesium contents of otosclerotic samples with abnormal crystalline structure in mg/100 mg bone In samples with abnormal crystal line structure the average content of calcium was 24 70 of phosphorus 14.27 of sodium 0.36 and of magnesium 0.23

Table 17 shows the constant weights and calcium phosphorus sodium and magnesium contents of otosclerotic samples with amorphous structure in mg/100 mg bone. In the samples with amorphous structure the average content of calcium was 18.24 of phosphorus 10.51 of sodium 0.35 and of magnesium 0 18

In Table 18 a comparison is carried out between the mineral contents of normal stapedial bone and otosclerotic stapedial bone with normal crystalline structure The calcium content of otosclerotic bone (25 85) is significantly lower than that of normal stapedial bone (30.28) ($p<0.01$) Correspond

Table 16 *Ca P Na and Mg contents of otosclerotic stapedial footplate in mg/100 mg bone. All samples represented abnormal diffraction patterns different from normal hydroxyapatite.*

Sample No	Weight (mg)	Ca	P	Na	Mg
1	0.521	21 1	13.3	0 17	0.20
2	0.282	22.0	14.3	0.25	0 11
3	0.372	22.0	12.7	0.20	0 15
4	0 484	36.8	21.3	0.24	0.23
5	0.200	20 0	12.0	0 40	0.20
6	0.221	22.2	12.7	0 49	0.20
7	0.279	21.5	13.6	0.57	0 45
8	0.264	36 9	18 4	0.29	0.34
9	0.281	21 4	11 4	0.52	0 17
10	0.606	23 1	13 0	0 45	0.27
Mean ($\bar{x}$)	0.351	24 70	14.27	0.36	0.23

Table 17 *Ca, P Na and Mg contents of otosclerotic stapedial footplate in mg/100 mg bone. All samples represented diffraction patterns typical of amorphous structure.*

Sample No.	Weight (mg)	Ca	P	Na	Mg
1	0.321	20.2	12.3	0.55	0 26
2	0.384	21 4	14.2	0 1[illegible]	0.07
3	1.548	21.0	10.6	0 45	0 23
4	0.224	17.9	11.8	0.09	0.07
5	0 122	18 4	11.6	0 17	0.08
6	1 054	19 7	9 7	0.37	0.25
7	0 114	5 4	3.5	0.59	0.04
8	1.340	19 4	9.9	0.40	0.22
9	1.280	18 4	9 9	0.35	0.23
10	0.884	20 6	11.6	0.42	0.34
Mean ($\bar{x}$)	0 730	18.24	10.51	0.35	0.18

Table 18 *Comparison between the Ca, P Na and Mg contents of normal stapedial footplate and otosclerotic bone with normal crystalline structure (=normal otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal stapes	30.28	18.03	3.34	0.01	38
	Normal otos. bone	25.85	13.25			
P	Normal stapes	15.96	4 64	3 17	0.01	38
	Normal otos. bone	13.87	3 74			
Na	Normal stapes	0 43	0.03	0.39	—	38
	Normal otos. bone	0 41	0.02			
Mg	Normal stapes	0.34	0 01	2.50	0.05	38
	Normal otos. bone	0.26	0.01			

Table 19 *Comparison bet\ een the Ca, P Na and Mg contents of normal stapedial footplate and otosclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal stapes	30.28	18.03	2.82	0.01	28
	Abnormal otos. bone	24.70	37.48			
P	Normal stapes	15.96	4.64	1.72	0.1	28
	Abnormal otos. bone	14.37	8.75			
Na	Normal stapes	0.43	0.03	1.08	—	28
	Abnormal otos. bone	0.36	0.02			
Mg	Normal stapes	0.34	0.01	2.75	0.01	28
	Abnormal otos. bone	0.23	0.01			

Table 20. *Comparison between the Ca, P Na and Mg contents of normal stapedial footplate and otosclerotic bone with amorphous structur (=amorphous otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone*

		$\bar{x}$	s^2	t	p	df
Ca	Normal stapes	30.28	18.03	6.96	0.001	28
	Amorphous otos. bone	18.24	19.55			
P	Normal stapes	15.96	4.64	6.18	0.001	28
	Amorphous otos. bone	10.51	7.14			
Na	Normal stapes	0.43	0.03	1.29	—	28
	Amorphous otos. bone	0.35	0.03			
Mg	Normal stapes	0.34	0.01	4.00	0.001	28
	Amorphous otos. bone	0.18	0.01			

Table 21 *Comparison between the Ca, P Na and Mg contents of otosclerotic bone with normal crystalline structure (=normal otosclerotic bone) and otosclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal otos. bone	25.85	13.25	0.62	—	28
	Abnormal otos. bone	24.70	37.48			
P	Normal otos. bone	13.87	3.74	0.43	—	28
	Abnormal otos. bone	14.27	8.75			
Na	Normal otos. bone	0.41	0.02	0.89	—	28
	Abnormal otos. bone	0.36	0.02			
Mg	Normal otos. bone	0.26	0.01	0.75	—	28
	Abnormal otos. bone	0.23	0.01			

ingly the phosphoru content of this kind of otosclerotic bone (13.87) is significantly lower than that of normal stapedial bone (15.96) ($p<0.01$) Between the sodium contents of normal stapedial bone (0.43) and otosclerotic bone with normal crystalline structure (0.41) no statistically significant difference was noted. On the other hand, the magnesium content of otosclerotic bone (0.26) is nearly significantly lower than that of normal stapedial bone (0.34) ($p<0.05$)

Table 22. *Comparison between the Ca, P Na and Mg contents of otosclerotic bone with normal crystalline structure (=normal otosclerotic bone) and otosclerotic bone with amorphous structure (=amorphous otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal otos bone	25.85	13.25			
	Amorphous otos bone	18.24	19.55	4.85	0 001	28
P	Normal otos. bone	13.87	3.74			
	Amorphous otos. bone	10.51	7 14	3.82	0.001	28
Na	Normal otos bone	0 41	0.02			
	Amorphous otos.bone	0.35	0.03	0 98	—	28
Mg	Normal otos bone	0.26	0.01			
	Amorphous otos bone	0.18	0.01	2.00	0.1	28

Table 23 *Comparison between the Ca, P Na and Mg contents of otosclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone) and otosclerotic bone with amorphous structure (=amorphous otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Abnormal otos bone	24 70	37 48			
	Amorphous otos bone	18.24	19.55	2.57	0.05	18
P	Abnormal otos. bone	14.27	8.75			
	Amorphous otos bone	10.51	7 14	2.83	0.05	18
Na	Abnormal otos. bone	0.36	0.02			
	Amorphous otos bone	0.35	0.03	0 14	—	18
Mg	Abnormal otos bone	0.23	0.01			
	Amorphous otos bone	0 18	0.01	1.06	—	18

When comparing the mineral contents of normal stapedial bone and otosclerotic stapedial bone with abnormal crystalline structure the calcium content in the last mentioned (24 70) is observed to be significantly lower than in the first mentioned (30.28) ($p<0.01$) On the other hand, the phosphorus content in the otosclerotic bone (14.27) does not statistically differ from that of normal stapedial bone (15 96) ($p<0\ 1$) In the sodium contents there is no statistically significant difference in this group either The magnesium content of otosclerotic bone (0.23) is again significantly lower than in the normal stapedial bone (0.34) ($p<0\ 01$) (Table 19)

In Table 20 a comparison is presented between the mineral contents of normal stapedial bone and otosclerotic stapedial bone with amorphous structure The calcium content is highly significantly lower in the otosclerotic group (18.24) than in the control group (30.28) ($p<0\ 001$) The same is true about the phosphorus content (10.51 and 15 96 respectively) ($p<0\ 001$) There is no statistically significant difference in th sodium contents but the magnesium content again (0 18) is highly significantly lower than in the normal bone (0.34) ($p<0\ 001$)

No statistically significant differences are noted between any mineral contents of otosclerotic bone with normal and with abnormal crystalline structures (Table 21)

In Table 22 there is a comparison between the mineral contents of otosclerotic stapedial bone with normal crystalline structure and with amorphous structure The calcium content is highly significantly lower in the amorphous *(18.24) than in normal (25.85)* structural group ($p<0\ 001$) The same is true

about the phosphorus content (10.51 and 13.87 correspondingly) ($p<0.001$) In the sodium and magnesium contents there are no significant differences between these groups.

In comparison between the mineral contents of otosclerotic bones with abnormal crystalline structure and with amorphous structure, it is noted that the calcium and phosphorus contents in the last mentioned group (18.24 and 10.51) are nearly significantly lower ($p<0.05$) No statistically significant differences are noted in the sodium and magnesium contents between these groups (Table 23)

Table 22. *Comparison between the Ca, P Na and Mg contents of otosclerotic bone with normal crystalline structure (=normal otosclerotic bone) and otosclerotic bone with amorphous structure (=amorphous otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

		$\bar{x}$	s^2	t	p	df
Ca	Normal otos bone	25.85	13.25			
	Amorphous otos. bone	18.24	19.55	4.85	0.001	21
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Table 23 *Comparison between the Ca, P Na and Mg contents of otosclerotic bone with abnormal crystalline structure (=abnormal otosclerotic bone) and otosclerotic bone with amorphous structure (=amorphous otosclerotic bone) The mean contents ($\bar{x}$) in mg/100 mg bone.*

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No statistically significant differences are noted between any mineral contents of otosclerotic bone with normal and with abnormal crystalline structures (Table 21)

In Table 22 there is a comparison between the mineral contents of otosclerotic stapedial bone with normal crystalline structure and with *amorphous* structure The calcium content is highly significantly lower in the amorphous *(18.24) than in normal (25.85) structural group ($p<0.001$) The same is true*

may effect somewhat the crystalline structure, so that estimation of results of a material treated in this way must be carried out carefully. On the other hand, treatment of the otosclerotic samples in +60°C water hardly had any effect on the bone crystals, though it is known that bone crystals usually grow in warm water. This is noted as sharpening of diffraction lines.

For the present investigation, the part of the stapedial footplate was chosen which looked most otosclerotic in the operating microscope. Similar morphologic grouping was used by Linck et al. (1967) in their study on the mineral content of the otosclerotic stapedial footplate. They divided the stapedial footplates into four groups, 0 %, 25 %, 50 % and 100 % otosclerotic samples. In addition it must be remembered that the patients from whom the samples had been taken had only clinically manifest otosclerosis. No histologic examination was performed so that it remains at least partly a suggestion that the samples really were otosclerotic. It is true that a part of the samples were examined by microradiography but the microradiographic and Debye-Scherrer samples were not exactly from the same location of the otosclerotic bone.

The mean age of the whole of the present material was 41.7 years, in the group with normal crystalline structure 41.7 years, with abnormal crystalline structure 39.4 years and with amorphous structure 42.4 years. There is no statistically significant differences in the mean ages of the groups. Neither are there statistically significant differences between the sexes. The same is true about the anterior and posterior parts of the stapedial footplate. Thus it seems evident that the different crystalline structures in this material are not dependent of the age, sex or original location of the sample, but the different structures are connected with the otosclerotic process itself.

In the present investigation no attemps were made to elucidate the finer structure of the deviating crystals. It was not possible to do it by the methods used. On the other hand, it must be kept in mind that investigators do not fully agree as to the crystalline structure of hydroxyapatite. The fact that no abnormal structures were found in Group C well agrees with the generally accepted view that in the upper parts of the stapedial crura or in the head of the stapes otosclerotic changes do not occur.

It is not correct to think that otosclerotic bone could generally be divided into different groups with normal and abnormal crystalline structures and amorphous structure, expressed in percentages. The results of this investigation mean of course that a certain amount of the bone needles examined belonged to each structural type. It is probable that if a sufficient number of bone needles would have been prepared of one and the same otosclerotic stapes Debye-Scherrer patterns showing both normal, abnormal and amorphous structures would have been obtained. Namely the fact is that all stages of the otosclerotic process can be found in one otosclerotic focus.

According to Oesterle (1933) cartilage may remain even in remarkable amounts in the bases of the stapedial crura for the whole life. This must also be kept in mind when the amount of samples with amorphous structure is analysed. It could be thought that the amorphous structure here would not represent otosclerotic bone but normal cartilage with no crystalline structure. In Altmann's (1962) opinion after the otosclerotic process has destroyed the cartilaginous covering of the window frame calcification occurs in the proliferated fibrous tissue, and thus is an ankylosing bridge formed at least in some cases in the stapedial footplate. This bridge is then resorbed while the actual otosclerotic bone grows in its place. On the other hand, it seems probable that the diffraction pattern of the calcified ankylosing bridge corresponds to that of hydroxyapatite. Diffraction patterns of calcifications

VI Discussion

A. X ray crystallographic examination

There are very few publications on crystallographic examinations of the normal auditory ossicles Meurman et al (1967 1968) published results of a study which was a preliminary report of the present investigation The present author examined the auditory ossicles of ten patients with no history of impaired hearing and found no diffraction patterns different from that of normal hydroxyapatite Similarly no abnormal crystallographic characteristics were noted in the bone pieces from the posterior meatal wall either in otosclerotic or healthy subjects. It is considered rather certain that the bone tissue of normal auditory ossicles as well as of normal and otosclerotic temporal bone does not differ from normal human bone as to the crystalline structure It must be remembered, however that histologic otosclerosis occurs in about 10 % of the population though only about 1 % has any clinical symptoms (Guild 1944 Larsson 1960 Altmann 1962) Thus it is presumable that histologic otosclerosis may occur in the so-called normal auditory ossicles The fact that there were no abnormal crystalline structures in the normal auditory ossicles may be due either to the real absence of otosclerotic changes in normal stapedes or absence of abnormal crystalline structures despite otosclerotic changes Nevertheless it can be thought that if there are otosclerotic changes in the stapedial footplate there are also changes in hearing Primary otosclerosis in the stapedial footplate is rather rare (Altmann 1962) It would have been interesting to examine the bone crystals of otosclerotic malleus and incus by the same method. This kind of material was however not available.

When the crystal orientation of the normal ossicles was examined by the Laue transmission method it was noted that the bone crystals in the handle of the malleus and long crus of the incus were generally arranged parallelly with the long axis The same is true about the anterior crus of the stapes but in the base of it there was great variability in the bone crystal orientation. It must be kept in mind that the crystal orientation examination based on x ray diffraction is only indicative and the complete rings in the Laue photograph do not necessarily mean absence of orientation (Glimcher 1959)

It is apparent however that the bone crystals in the auditory ossicles are oriented in the same way as in other bones i.e., the crystals follow the direction of collagen fibrils Chevance (1961) and Reydon & Smith (1968) observed remarkable variability in collagen fibril arrangement of the stapedial footplate which well agrees with the results of the present investigation

The otosclerotic bone samples of the present series consisted of pieces of stapedes which had been removed at operations for otosclerosis The small size of the samples made it necessary to use thin bone needles instead of powder specimens in the Debye-Scherrer examination Another possibility would have been to mix the powder from the specimens with glue and draw it out to fine fibers The needles were attempted to be made as thin as possible because the compact bone ought to be about 75 μm thick in order to obtain an optimal diffraction photograph (Carlström 1955) In order to eliminate the disturbing background scattering caused by water and the organic component of the bone a part of the samples were extracted by ethylendiamine Deproteinization

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It is presumed that in the beginning of the otosclerotic process, bone crystals are not observed at all (amorphous structure). Gradually small bone crystals develop the diffraction lines are diffuse and broad. Little by little the crystals grow and reach at the sclerotic stage a size which is not observed in normal bone. It is obvious that in addition to the size, there are other structural changes as well, which are seen as distinct alterations in the diffraction lines.

Attention must be paid to the fact, which was proved by Termine and Posner (1967) that a remarkable part of the mineral content of normal bone may be amorphous calcium phosphate. Termine & Posner suggested furthermore that amorphous calcium phosphate be the first mineral deposited during the calcification process. Fleisch et al. (1964) noted that inorganic pyrophosphate markedly increased the time required for the transformation of amorphous to crystalline calcium phosphate. They proposed that pyrophosphate may be one of the factors that allows a part of the bone mineral to persist in a non-crystalline state. The alkaline phosphatase might be able to accelerate the transformation process. These circumstances must be remembered when the different crystalline structures and their percentages are analysed in this investigation.

Maurer (1967) suggested that the apatite—fibril relationship is changed in otosclerotic bone. In their electron microscope examination Frank et al. (1964) observed small bone crystals in the otospongiotic phase which will agrees with the results of the present investigation. In addition, Frank et al found big crystals even as long as 3 000 Å, but in osteocytic sclerosis only. They did not find crystal of this length in normal bone but certainly in dentinal sclerosis. Furthermore, they suggested that big single crystal develop from octocalcium phosphate. Octocalcium phosphate is known to alter hydroxyapatite e.g., in electron beam. They also proposed that the osteocytic sclerosis is able to alter the vibratory transmission properties of the stapes.

Linck et al. (1967) noted that otosclerotic bone contains as many as three times so much fluoride as meatal bone. On the other hand, it is known that influenced by fuorine apatite crystals are larger more perfect and less soluble (Eanes 1965). It is known though, that if there is a surplus amount of Mg^{++} ions they fill the lattice surface and the crystal growth is ceased. Francis (1969) stated that both polyphosphonates and polyphosphates are efficient inhibitors of hydroxyapatite crystal growth. Furthermore it has been found out that young bone has a wider surface in contact with the surrounding fluid, so that it is more sensitive to the influences of the environment than old bone. It would be interesting to know whether these observations have any connection with the pathologic crystalline structures found in the present investigation.

According to Frost (1962) no distinct orientation of lacunae and canaliculi can be seen in the otosclerotic bone. This agrees with the result of the present Laue examination that at least in a part of otosclerotic foci, bone crystal orientation is missing. On the other hand, the results of the Laue examination can be considered indicative only. Thus, e.g., if the c-axis of bone crystals is parallel to the x-ray beam no "arcing" phenomenon is present though the bone crystals would in fact be oriented. This possibility is rare, though. Lack of preferred orientation is typical not only of otosclerotic bone, because similar arrangement of bone crystals is met in fibrous bone as well, which is present in many bone diseases elsewhere than in the labyrinthine capsule. In crystallizing cartilage as well bone crystals are arranged at random (Robinson & Cameron 1956). The same is true about embryonic bone (Clarc & Iball 1957). All these observations indicate that at its initial stage, otosclerotic bone is young, rapidly developing fibrous bone (Reydon & Smith 1968).

in blood vessel walls correspond to those of hydroxyapatite (Carlström 1955) and so do diffraction patterns of tympanosclerosis (the author's observation)

Three of the diffraction patterns of samples treated with ethylendiamine were abnormal, whereas a normal hydroxyapatite pattern was obtained of the corresponding untreated samples. It is not quite certain, whether the crystalline structures were really pathologic or whether the change was due to deproteinization treatment

When analysing the results, it must be remembered carefully that in the otosclerotic stapes there are at the same time and next to each other areas with quite normal ultrastructure and areas with different otosclerotic changes (Frank et al 1968) Similarly there are otosclerotic foci of different stages side by side in the footplate and these foci develop independently of each other On the other hand the development of the otosclerotic focus may stop and then start again (Hall & Ogilvie 1963) Furthermore otosclerotic changes may be local and diffuse.

Weber observed as early as in 1931 that the collagen fibrils in otosclerotic focus clearly differ from the fibrils outside the focus Chevance (1962) also found different changes in the collagen fibrils of the focus Other investigators (Reydon & Smith 1968) as well have noted changes in the collagen of otosclerotic bone. Soifer et al (1969 b) could not find any abnormal changes in the total amount of collagen in the otosclerotic stapes. Thus the changes are probably qualitative. Frost (1962) thinks that the primary factor in otosclerosis is some change in the cement substance of the labyrinthine capsule What makes osteoblasts produce abnormal cement substance is still unsolved Maurer (1962) thought that generally in the area of fibrous bone the organic component is different from other parts of bone tissue Ardouin & Wegmann (1961) noted disturbances in mucopolysaccharides and polysaccharides in the otosclerotic focus In their investigation Arslan & Ricci (1961) found alterations in the osteomucoid substance Soifer et al (1969b) could not find any changes in the total amount of mucopolysaccharides so that changes seem to be in the different components. Chevance et al (1961 b) and Soifer et al (1969 a) noted that during the active phase of the otosclerotic process, the activity of alkaline phosphatase in the focus has increased. The same phenomenon was observed by Ricci (1962) too As well numerous other enzyme activities have been noted to increase during the active phase of the otosclerotic process (Albernaz & Covell 1961 Ardouin & Wegmann 1961) Soifer et al (1969 a) found however that increase in the alkaline phosphatase and decrease in the lactate dehydrogenase activities alone are statistically significant All these observations indicate abnormal changes in the organic component of the otosclerotic focus. In normal bone, the crystals are arranged in line with collagen fibrils and it is proposed that there are apparently three crystallites for each 640 Å period of the collagen fibril Some kind of a connection has been noted between the crystal size and repeating units of the collagen fibrils. In the light of these investigations, a very interesting observation was made by Reydon & Smith (1968) They found that in the otosclerotic stapes the dense band in the collagen period appeared wider than that in bone collagen from normal stapes

On the basis of the present investigation it is obvious that besides the normal hydroxyapatite crystalline structure there are also abnormal crystalline structures in the otosclerotic focus. In addition fibrous tissue is apparently present in which distinct crystal formation has not taken place yet That is why this tissue gives a diffraction pattern typical of the amorphous structure In a part of the photographs, the lines are broad and ill-defined which indicates that most of the crystals are small in their siz

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Weber observed as early as in 1931 that the collagen fibrils in otosclerotic focus clearly differ from the fibrils outside the focus. Chevance (1962) also found different changes in the collagen fibrils of the focus. Other investigators (Reydon & Smith 1968) as well have noted changes in the collagen of otosclerotic bone. Soifer et al (1969 b) could not find any abnormal changes in the total amount of collagen in the otosclerotic stapes. Thus the changes are probably qualitative Frost (1962) thinks that the primary factor in otosclerosis is some change in the cement substance of the labyrinthine capsule What makes osteoblasts produce abnormal cement substance is still unsolved Maurer (1962) thought that generally in the area of fibrous bone the organic component is different from other parts of bone tissue Ardouin & Wegmann (1961) noted disturbances in mucopolysaccharides and polysaccharides in the otosclerotic focus In their investigation Arslan & Ricci (1961) found alterations in the osteomucoid substance. Soifer et al (1969b) could not find any *changes in the total* amount of mucopolysaccharides so that changes seem to be in the different components. Chevance et al (1961 b) and *Soifer* et al (1969 a) noted that during the active phase of the otosclerotic process the activity of alkaline phosphatase in the focus has increased. The same phenomenon was observed by Ricci (1962) too As well numerous other enzyme activities have been noted *to* increase during the active phase of the otosclerotic process (Albernaz & Covell 1961 Ardouin & Wegmann 1961) Soifer *et* al. (1969 a) found however that increase in the alkaline phosphatase and decrease in the lactate dehydrogenase activities alone are statistically significant. All these observations indicate abnormal changes in the organic component of the otosclerotic focus. In normal bone the crystals are arranged in line with collagen fibrils and it is proposed that there are apparently three crystallites for each 640 Å period of the collagen fibril Some kind of a connection has been noted between the crystal size and repeating units of the collagen fibrils. In the light of these investigations a very interesting observation was made by Reydon & Smith (1968) They found that in the otosclerotic stapes the dense band in the collagen period appeared wider than that in bone collagen from normal stapes"

On the basis of the present investigation it is obvious that besides the normal hydroxyapatite crystalline structure there are also abnormal crystalline structures in the otosclerotic focus In addition, fibrous tissue is apparently present in which distinct crystal formation has not taken place yet. That is why this tissue gives a diffraction pattern typical of the amorphous structure In a part of the photographs the lines are broad and ill-defined which indicates that most of the crystals are small in their size.

It is presumed that in the beginning of the otosclerotic process, bone crystals are not observed at all (amorphous structure). Gradually small bone crystals develop, the diffraction lines are diffuse and broad. Little by little the crystals grow and reach at the sclerotic stage a size which is not observed in normal bone. It is obvious that in addition to the size, there are other structural changes as well which are seen as distinct alterations in the diffraction lines.

Attention must be paid to the fact, which was proved by Termine and Posner (1967) that a remarkable part of the mineral content of normal bone may be amorphous calcium phosphate. Termine & Posner suggested furthermore that amorphous calcium phosphate be the first mineral deposited during the calcification process. Fleisch et al. (1968) noted that inorganic pyrophosphate markedly increased the time required for the transformation of amorphous to crystalline calcium phosphate. They proposed that pyrophosphate may be one of the factors that allows a part of the bone mineral to persist in a non-crystalline state. The alkaline phosphatase might be able to accelerate the transformation process. These circumstances must be remembered when the different crystalline structures and their percentages are analysed in this investigation.

Maurer (1967) suggested that the apatite—fibril relationship is changed in otosclerotic bone. In their electron microscope examination, Frank et al. (1968) observed small bone crystals in the otospongiotic phase which well agrees with the results of the present investigation. In addition, Frank et al. found big crystals even as long as 3 000 Å but in osteocytic sclerosis only. They did not find crystals of this length in normal bone, but certainly in dentinal sclerosis. Furthermore, they suggested that big single crystals develop from octocalcium phosphate. Octocalcium phosphate is known to alter hydroxyapatite, e.g., in electron beam. They also proposed that the osteocytic sclerosis is able to alter the vibratory transmission properties of the stapes.

Linck et al. (1967) noted that otosclerotic bone contains as many as three times so much fluoride as meatal bone. On the other hand, it is known that influenced by fluorine apatite crystals are larger, more perfect and less soluble (Eanes 1965). It is known though, that if there is a surplus amount of Mg^{++} ions they fill the lattice surface and the crystal growth is ceased. Francis (1969) stated that both polyphosphonates and polyphosphates are efficient inhibitors of hydroxyapatite crystal growth. Furthermore it has been found out that young bone has a wider surface in contact with the surrounding fluid, so that it is more sensitive to the influences of the environment than old bone. It would be interesting to know whether these observations have any connection with the pathologic crystalline structures found in the present investigation.

According to Frost (1962), no distinct orientation of lacunae and canaliculi can be seen in the otosclerotic bone. This agrees with the result of the present Laue examination that at least in a part of otosclerotic foci, bone crystal orientation is missing. On the other hand, the results of the Laue examination can be considered indicative only. Thus, e.g., if the c-axis of bone crystals is parallel to the x-ray beam no "arcing" phenomenon is present though the bone crystals would in fact be oriented. This possibility is rare though. Lack of preferred orientation is typical not only of otosclerotic bone, because similar arrangement of bone crystals is met in fibrous bone as well, which is present in many bone diseases even elsewhere than in the labyrinthine capsule. In crystallizing cartilage as well, bone crystals are arranged at random (Robinson & Cameron 1956). The same is true about embryonic bone (Clarc & Iball 1957). All these observations indicate that at its initial stage otosclerotic bone is young, rapidly developing fibrous bone (Reydon & Smith 1968).

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B. Microradiographic examination

Karlsson et al (1954) and Orlandini & Sbernini (1956) have studied normal auditory ossicles employing microradiographic methods In the present investigation this was not done the primary aim being to determine the mineral content of otosclerotic bone by quantitative microradiography Nevertheless otosclerotic bone in its active phase differs so much from normal bone that the traditional microradiographic methods were difficult to apply First of all the strong porosity of active otosclerotic bone caused difficulties This was also noted by Garcia Ibañez (1968) In the present investigation an additional source of error was the fact that the methylmethacrylate sections were not plane parallel Furthermore determination of the section thicknesses was inaccurate Because of these reasons the conclusions drawn by quantitative microradiography were only indicative.

The developmental stage of the otosclerotic bone was tried to be determined by microradiographs The present author wanted to obtain suggestions at what developmental stage of otosclerosis varying crystalline structures observed in the Debye-Scherrer analysis occur and whether they are in any way connected with the otosclerotic process In estimation of the developmental stage traditional histologic methods would obviously have been more useful (Henner et al 1960) Because of the great number of methods and scarcity of material it was necessary to limit the investigation to the microradiographic examination The same methylmethacrylate sections had previously been used in the Laue examination

Fifty otosclerotic bone samples were studied by microradiographic methods 30 of them had normal and 10 abnormal crystalline structure and 10 had amorphous structure. All samples were either from the anterior part of the stapedial footplate or macroscopically distinct separate otosclerotic foci Thus the material was selected up to a great extent and it cannot be compared with unselected otosclerotic stapes material The material was grouped according to which type of the process was the most dominating

In the pertinent literature there are several principles according to which the otosclerotic process is divided. Nylén (1949) called the different types local and diffuse otosclerosis In his material 10 % was diffuse. The foci were active (20 %) quiescent (30 %) and mixed (50 %) Ogilvie & Hall (1953 1962) used the division into local and diffuse as well and the developmental stages were called by them active quiescent healing and healed Ludman (1962) again divided his otosclerotic material as follows 1 early focal (10 %) 2 active diffuse (38 %) 3 quiescent (52 %) Ludman suggested two different types of clinical otosclerosis. Wolff & Bellucci (1964) divided the otosclerotic changes into six types They also emphasized that division of the samples is very difficult because under the microscope it is possible to see several types and stages of the disease in the view of field at one and the same time. Gukovich (1964) used the following division 1 immature foci (high activity) 2 foci with medium degree of maturity (moderate activity) 3 mature foci (low activity) When attention is also paid to the fact that no distinct lines can be drawn between the different types and stages presenting of percentages is greatly based on the investigator s subjective opinion

As has been mentioned earlier the aim of the microradiographic examination was to elucidate the occurrence of the three crystalline structures at various developmental stages of otosclerosis Certain conclusions were not possible to draw because the bone sample for the Debye-Scherrer analysis was not from exactly the same place in the original preparation as the sample for the microradiographic examination. It is possible that both sample groups included oto-

sclerotic bone material of quite a different stage and type. That is why the distribution in percentages must be analysed with reservation. Nevertheless it is very probable that the samples which were classified amorphous on the basis of the Debye-Scherrer analysis represent the youngest and most active otosclerotic process, in which bone crystals have not developed yet or are still very small and imperfect. On the other hand, with regard to the samples with abnormal crystalline structure, the conclusion is not so clear because they can obviously appear both in younger and in older inactive otosclerotic bone.

C. Quantitative mineral analysis

In the present investigation, the phosphorus and sodium contents were determined by neutron activation analysis and the calcium and magnesium contents by atom absorption spectrophotometry. The methods can be considered relatively accurate at least for calcium and phosphorus because the determination accuracy of these elements was estimated about ± 3 %. For sodium the accuracy was ± 5 % and for magnesium it was only ± 10—20 %. The small size of the samples caused difficulties, but excluding some individual cases the results can be considered reliable.

Samples of the anterior part of the stapedial footplate were chosen as control material because the otosclerotic material also represented the anterior part of the footplate or separate otosclerotic foci. It must be kept in mind, as has been earlier mentioned, that in the bases of the stapedial crura, there may be an abundance of cartilage (Oesterle 1933). This may cause false results in the analyses both of control and otosclerotic samples. The mineral analysis results of calcium in control material rather well agree with the average calcium content of 30.0 % obtained by Maurer (1960). Maurer (1967) continued his investigations, and measured the corresponding figure 29.4 %. The value for phosphorus in the present investigation 15.96 % is higher than the percentages by Maurer (13.7 % in 1960 and 13.7 % in 1967). This variation may be due to differences in the measuring techniques.

The following values were obtained in normal stapes by Soifer et al. (1970 a): calcium 29.2 % and phosphorus 11.1 % and by Soifer et al. (1970 b): calcium 28.6 %, sodium 0.766 % and magnesium 0.325 %. It must be remembered, however, that both Maurer and Soifer et al. used whole stapedes in their measurements.

The otosclerotic material in the mineral analysis was selected, so that it is difficult to compare these results with the earlier ones. In the first place, comparisons could be carried out with otosclerotic material with normal crystalline structure but not even this group is completely comparable with unselected otosclerotic material. Furthermore all measurement results have been obtained either from the anterior part of the otosclerotic stapedial footplate or a separate otosclerotic focus. In other words from the most otosclerotic locations, whereas Maurer (1967) and Soifer et al. (1970 a, 1970 b) used whole stapedes also in their analyses of otosclerotic material. Maurer (1967) emphasized that the stapedes in his material did not include at least macroscopically observable otosclerotic foci, whereas it was expressly aimed at in the present investigation. Nevertheless, when the bone samples are smaller also measuring errors grow. In addition, in one and the same bone, mineral contents may vary a great deal even in a very small area. A more descriptive method would obviously be to determine the mineral contents per unit volume because the structure of otosclerotic bone would then be better revealed (Röckert et al. 1965). Maurer (1967) found the average calcium content of otosclerotic stapes to be 29.2 % and the average phosphorus content 13.4 %. Soifer et al. (1970 a)

obtained the corresponding values 28.4 % and 11.0 % and Soifer et al (1970 b) for calcium 27.5 % magnesium 0.327 % and sodium 0.869 %. Soifer et al (1970 a) could not find a correlation on one hand between the calcium and phosphorus contents and on the other hand between the sex age or otosclerotic growth in footplates.

It is interesting to compare the mineral contents between the different otosclerotic groups of the present investigation and normal stapedial footplate. Maurer (1967) observed that the calcium and phosphorus contents of the otosclerotic stapes were significantly lower than the corresponding values of the normal stapes. On the other hand Soifer et al (1970 a) did not note a statistically significant difference between corresponding groups. Linck et al (1967) found out that even a 100 % otosclerotic stapedial footplate contained less calcium than a normal one. It was observed in the present investigation that the calcium and phosphorus contents of otosclerotic bone with normal crystalline structure were significantly lower than those of normal stapedial bone. No difference was noted between the sodium contents and the magnesium content was nearly significantly lower.

Between otosclerotic samples with normal and abnormal crystalline structures no statistically significant differences were noted for any of the minerals examined. Neither were there any clear differences between these groups in the microradiographic examination. It is quite obvious that these two groups need not be separated and they do not necessarily represent different phases in the otosclerotic process. On the basis of the present results it seems also apparent that crystalline structures deviating from the normal hydroxyapatite may occur as well in young rather active as in older inactive otosclerotic foci.

Amorphous otosclerotic bone however clearly differs from normal otosclerotic bone with regard to its minerals. Both calcium and phosphorus contents in otosclerotic bone were in this group highly significantly lower than those of the normal stapes or otosclerotic bone with normal crystalline structure. On the other hand the sodium and magnesium contents did not differ from the corresponding values of normal otosclerotic bone. This mineral analysis supports the earlier suggestion that amorphous otosclerotic bone represents a young, active focus whose mineral content is low and bone crystals have not yet developed properly.

In order to find out whether otosclerosis is a local or more generalized process changes in the mineral contents of serum have been examined but the results have been partly conflicting. As well alkaline phosphatase activity in sera from otosclerotics have been determined and possible correlations looked for in it but even these results have turned out somewhat conflicting. Determination of different hormones and their catabolites has been attempted to be elucidated knowing the connection between hormones and bone metabolism but no certain conception of the hormonal status of otosclerotic patients has been obtained. Vysloužil's (1961 1963) observations on high estrogen levels in otosclerotics seem somewhat more reliable.

Maurer (1967) drew the conclusion that in otosclerosis the fibrillar content is higher than normally in areas consisting mainly of web-like bone. He deduced from this that the otosclerotic process is not only local. True Pedersen & Sörensen (1970) examined normal and otosclerotic stapedial crura in light and electron microscopy but could find no differences in the periosteum or bone tissue. This again speaks against a general ized connective tissue disease. On the other hand observations on lower mineral contents than normally in the malleus and incus in connection with otosclerosis indicate a more generalized process (Mello & Sillingardi 1952 Flor & Martinozzi 1960 Evens &

Henkin 1969) It must be remembered however that alterations in the mineral contents of all auditory ossicles may be secondary changes resulting from stapedial ankylosis in other words, osteoporosis caused by impaired motility No certain conclusion can however be drawn whether otosclerosis is merely a local disease of the labyrinthine capsule or a local manifestation of a more generalized disorder

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VII Summary

The aim of the study was to investigate by the Debye Scherrer method the crystalline structures of normal auditory ossicles and otosclerotic bone by comparing them with the crystalline structure of normal human bone. In addition crystal orientation in normal auditory ossicles and otosclerotic bone was investigated by the Laue transmission method. By virtue of the microradiographic examination the present author attempted to find out at what developmental stage of otosclerosis alterations in the crystalline structure appear. Finally the mineral contents of the normal stapes and different otosclerotic foci observed by the x-ray crystallographic examination were studied. The calcium and magnesium contents were determined by atom absorption spectrophotometry and the phosphorus and sodium contents by neutron activation analysis.

The auditory ossicles of 10 deceased with no history of impaired hearing were examined by the Debye-Scherrer method. Powder patterns were made of seven different locations of the ossicular chain. All diffraction patterns were identical with that of normal human bone. As well the diffraction patterns of the posterior meatal wall of controls and otosclerotics were identical with the pattern of normal hydroxyapatite.

The auditory ossicles of 10 deceased with no history of impaired hearing were examined by the Laue transmission method. It was noted that the bone crystals were arranged parallelly with the collagen fibrils. In the stapedial footplate however great variation was noted in the bone crystal orientation.

The otosclerotic material consisted of 250 samples obtained in connection with operations for otosclerosis. They were pieces of otosclerotic stapedes or separate otosclerotic foci. In the Debye-Scherrer examination, a diffraction pattern of normal hydroxyapatite was obtained in 75 %, an abnormal pattern in 7 % and a pattern typical of amorphous structure in 18 %. As to the stapedial foot plate alone in 73 % of the 237 samples examined the diffraction pattern was that of normal hydroxyapatite, 8 % gave an abnormal pattern and 19 % a pattern showing amorphous structure. The patterns of the different crystalline structures did not correlate in any way with the sex or age of the patients or original location of the sample.

Bone crystal orientation was examined in 50 otosclerotic samples. In 24 % a more or less preferred orientation was noted whereas no preferred orientation was seen in 68 %. In the samples with the normal crystalline structure preferred orientation was observed in 25 %, whereas in the samples with the abnormal crystalline structure preferred orientation was seen in 20 % and no preferred orientation in 80 %.

Fifty otosclerotic samples were examined by the microradiographic technique. The developmental stage of the otosclerotic process was thus attempted to be elucidated. Grouping by Henner et al. (1960) was employed. Out of the samples with normal crystalline structure 30 % represented the fourth stage, 30 % the third, 17 % the second and 23 % the first. As to the samples with an abnormal crystalline structure 30 % belonged to the fourth stage, 40 % to the third, 20 % to the second and 10 % to the first. All samples with amorphous structure belonged to the fourth developmental stage.

The mineral contents were determined from the anterior part of 20 normal staped-

ial footplates. The average calcium content was 30.28 phosphorus 15.96 sodium 0.43 and magnesium 0.34 mg/100 mg bone.

Forty otosclerotic samples were selected for the mineral analysis. In the group with the normal crystalline structure the average calcium content was 25.85 phosphorus 13.87 sodium 0.41 and magnesium 0.26 mg/100 mg bone. In the group with an abnormal crystalline structure, the average calcium content was 24.70 phosphorus 14.27 sodium 0.36 and magnesium 0.23 mg/100 mg bone. In the group with amorphous structure the average contents were as follows for calcium 18.24 phosphorus 10.51 sodium 0.35 and magnesium 0.18 mg/100 mg bone.

The calcium and phosphorus contents in the otosclerotic group with the normal crystalline structure were statistically significantly lower than those of the normal stapedial footplate, and the magnesium content was nearly significantly lower In the group with the abnormal structure as well the calcium and magnesium contents were significantly lower Between the otosclerotic samples with normal and abnormal structures no significant differences were noted for any of the minerals. On the other hand, the calcium and phosphorus contents in the otosclerotic samples with amorphous structure were highly significantly lower than those of the normal stapedial footplate or otosclerotic bone with the normal crystalline structure.

The present investigation reveals that otosclerotic bone with amorphous structure represents the youngest, most active otosclerotic focus, in which bone crystals have not developed yet or in which they are very small. Similarly the mineral contents in an otosclerotic focus of this type are clearly lower than in otosclerotic bone with the normal crystalline structure. On the other hand, abnormal crystalline structures obviously appear even at other otosclerotic stages, and this kind of otosclerotic bone hardly differs from normal otosclerotic bone It is concluded that abnormal crystalline structures are apparently caused by alterations in the organic component of otosclerotic bone.

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BY

J DELARUELLE and J MARQUET



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BY

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Abstract

This is a method of anaesthesia wich produces a deep, residual analgesia and neurolepsia, in which the activity of the autonomic nervous system is markedly reduced. Non-depressed, completely spontaneous respiration is to be compared with that seen in normal subjects at night during a period of so-called deep sleep ("Protected Sleep") In our opinion, this is a factor of great importance, enabling the preservation of (1) a constant pCO_2, (2) a central and peripheral circulation without any vasoconstrictions, and (3) a constant and low venous pressure, in the operative as well as in the postoperative phase, without any sudden transition.

This state is further characterized by a relative arterial hypotension, a good peripheral and tissue circulation and a mild hypothermia. In this way we can obtain a surgical field with minimal bleeding, no oozing and with good conditions for operating in ENT surgery (especially microsurgery of the ear), neurosurgery ophthalmic surgery and maxillo-facial surgery

The postoperative period is characterized by a calm and gradual awakening. The method is described on a clinical and practical basis. Theoretical considerations are being kept to a minimum and included only in order to clarify particular aspects.

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A General Aspects of the Technique

1 Arterial Hypotension

Before deliberately inducing even a moderate degree of arterial hypotension, one should always take care and make sure *that no harm* whatsoever might result (Rollason & Hough, 1960 Rollason, 1965 Slack & Walther 1964 Larsen, 1964 Conley Hincks & Jasaitis, 1965). In our opinion, induced arterial hypotension carries risks in application when one or more of the three following circumstances are present.

(*a*) a relative cellular hypoxia and insufficient tissue perfusion (a theoretically *unavoidable* consequency *of respiratory alkalosis and thus* of controlled hyperventilation (Andrew & Roth, 1968 Rengachery Roth, Andrew & Mark, 1967 Wollman, Craighead, Cohen, Smith, Chase & van der Molen, 1965 Keuskamp, 1965 Wollman, Smith, Stephen, Colton, Gleaton & Craighead, 1968 Tindall, Craddock & Greenfield, 1967)

(*b*) *an insufficient suppression* of neuro-vegetative activity with still possibility of vivid adrenergic baroreceptor and other reflexes with the consequent possibility of important peripheral vasoconstrictor reactions. That is to say too light anaesthesia and insufficient analgesia (Williams & Jones, 1968).

(*c*) a enous pressure not stabilized at a slightly lower value than preoperatively

These are the principal reasons why we abandoned controlled ventilation and light anaesthesia and searched for a spontaneous, non-depressed respiration together with a deeper degree of anaesthesia with sufficient depression of autonomic nervous activity

2. Spontaneous Respiration and Deep Narco-neuroleptanalgesia

The described anaesthetic technique permits a *completely* spontaneous respiration comparable to that of a normal subject at night during a period of so-called, deep-sleep Protected Sleep

The average values for the pCO_2 and the pH illustrate the same phenomenon (Comroe, 1966) (see further in Control Investigations). This can be achieved by means of the combination of drugs we use and which will be discussed *later*

Keeping in mind that CO is the only physiological vasodilatator (Molnar & Seylaz, 1967 Loew Palleske & Herrmann, 1967) it would seem preferable not to wash out the normal CO_2 stores of the body by means of artificial ventilation, when one is not really obliged to do so. On the contrary it would seem desirable, physiologically to maintain a fairly constant pCO_2, even slightly more on the acid side, *close to the limits of normal values but without* metabolic acidosis.

Sufficiently deep anaesthesia and maintenance of an adequate spontaneous respiration will guarantee a central and peripheral circulation, without vasoconstriction. Consequently a nearly normal state in the capillary circulation, in tissue perfusion and in tissue oxygenation is achieved.

At the same time we are able to avoid postoperative *hypoventilation* and *hypoxia* which normally occur after a classic *hyperventilation* technique. This period of hypoventilation with respiratory CO_2 retention occurs in order *to*

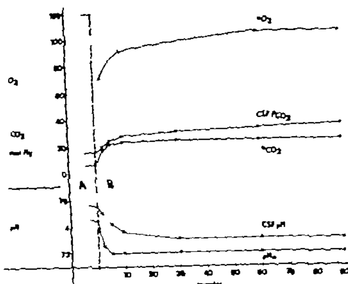


Fig. 2. Arterial and cisternal CFS gas tensions and pH during spontaneous recovery from hyperventilation. Period *A* hyperventilation with air for 60 min. Period *B* mechanical ventilation discontinued, allowing spontaneous return of breathing. Sullivan, S. F et al. (Ibid.), 1967

Nalorphine, muscular rigidity or any other extrapyramidal symptoms. With this drug we are able to obtain the constant, low venous pressure that is our goal. This low venous pressure is a result of the normo-physiologic venous return to the right heart during spontaneous inspiration, together with arterial hypotension and dilated capillary bed. Moreover this drug possesses analgesic properties of its own (Courvoisier & Leau, 1959 Lasagne & De Kornfeld, 1961 Maxwell, Palmer & Ryall, 1961 Paradis, 1962 Montilla, Frederik & Cass, 1965 Bloomfield, Simard-Savoie, Bernier & Tetreault, 1964 Kast & Collins, 1966 Beaver Wallenstein, Houde & Rogers, 1966 Council on Drugs, 1968).

Some of the recently developed active synthetic analgesics have been tried. They have not proved ideal for our purpose when used in large doses, since they produced a marked depression of respiration (Benzer Brunner Lempert & Muhar 1967 Benzer Brunner Lempert, Muhar & Pall, 1968).

Neither was simultaneous administration of a certain dose of Nalorphine a success. In fact, when using these drugs, artificial ventilation is an absolute necessity and for our purpose we do not want this unphysiological respiration.

For this reason we have returned to the use of Pethidine. This synthetic analgesic is fairly well-known (Goodman & Gilman, 1965), it is not too potent and permits simultaneous administration of Nalorphine (Goodman & Gilman, 1965). In a proportion of 10 mg N Allyl-Normorphine to 300 to 400 mg Pethidine adequate spontaneous respiration is maintained (Foldes, Schapira, Tarda, Duncalf & Shiffman, 1965 Foldes & Tarda, 1965). In this way one of the most outstanding side effects of this morphinomimetic drug is suppressed. Consequently it is possible to give a much larger dose of Pethidine and thus achieve a comfortable analgesic and hypnotic action.

In the intramuscular premedication we may also include Promethazine (Courvoisier Ducrot & Julou, 1957 Kinross-Wright, 1967) to reinforce hypnosis and Diazepam (Brandt & Cakes, 1965 Jaquenoud, 1965 Tornetta, 1965 Touchard & Bobin, 1966 Beaulieu, Boyette, Keeri-Saanto & Rheault, 1967) to reinforce the degree of general relaxation. In the doses used, these drugs do not depress spontaneous respiration (Steen, Weitzner Amaha & Martinez, 1966).

In 1969 we also used Diazepam as an intravenous induction agent, instead of thiopentone, so far with excellent results (Dalen, Evans, Banas, Brooks, Paraskos & Dexter

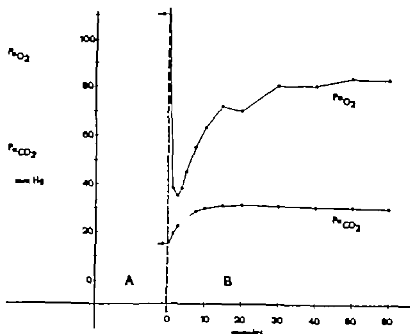


Fig 1 Posthyperventilation blood gas tensions. Period *A* represents steady hyperventilation with air for 60 min. Period *B* mechanical ventilation discontinued, allowing spontaneous return of breathing.
Sullivan, S. F., Patterson R. W & Papper E. M Department of Anesthesiology College of Physicians and Surgeons, Columbia University New York City Posthyperventilation hypoxia. *J Appl Physiol 22* (3) 431 5 1967

restore the depletion of CO_2 stores of the body (Salvatore, Sullivan & Papper 1969 Markello, Cutter & King, 1963 Marshall & Millar 1965) (Figs. 1 and 2) Furthermore, it is also possible to obtain a constant and low venous pressure during the operative, as well as the immediate postoperative periods, without any sudden transition This is mainly due to the normal physiological venous return to the right heart and which flows unchanged and unhindered *during spontaneuos inspiratory thoracic expansion.*

In these circumstances even with a certain degree of arterial hypotension, *the normal gradient of the tissue blood flow will be maintained*

A better surgical field is produced with fewer postoperative complications, whilst the induced state will return only very gradually to the preoperative state

With all this in mind we no longer use artificial ventilation for surgery of the head and neck In fact this kind of surgery by itself does not require curarization or a similar state, but a surgical field with minimal bleeding and free from vascular congestion (Conley Hincks & Jasaitis 1965) Our anaesthetic equipment has been reduced to a minimum whilst the pharmacodynamic part of anaesthesia has increased in importance.

3 Pharmacodynamic Medication

In order to obtain the anaesthetic state of Protected Sleep" we use a pharmacodynamic mixture, based on the association of the following drugs. Pethidine (Meperidine) N Allyl Normorphine, and Levomepromazine (Methotrimeprazine) *(P N.L. mixture) The choice of this* medication was made only after the most careful clinical tests, carried out with other known neuroplegics and analgesics.

In order to achieve the desired degree of neurolepsia we are at present using Levomepromazine, a phenothiazine derivate This drug possesses interesting autonomic nervous depressing effects, orthosympathicolytic and parasympathicolytic *(Julou Ducrot & Fouche,* 1966 Courvoisier Ducrot, Fournel & Julou 1957 Courvoisier Ducrot & Julou, 1957 Kinross-Wright 1967 Laborit, 1959 Du Cailar 1961 Decourt 1959 Dobkin, Levy Ounkasem & Pieloch 1968).

Besides, even very high doses do not interfere with spontaneus respiration. *Nor have* we observed, in combination with Pethidine–

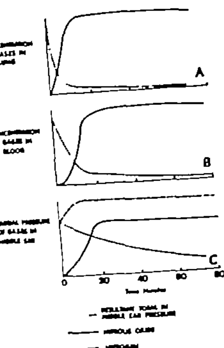


Fig. 3 (*A*) show rapid increase of pulmonary nitrous oxide with concomitant decrease in nitrogen concentration. This is associated (*B*) with similar changes in blood. This results in rapid increase of nitrous oxide partial pressure in the middle ear associated with gradual decrease in the partial pressure of nitrogen, resulting in an increase in middle ear pressure (*C*).

Matz, G. J. Rattenborg, C. G. & Holaday, D. A. Section of Otolaryngology and of Anesthesiology University of Chicago School of Medicine, Chicago. Effects of nitrous oxide on middle ear pressure. *Anesthesiology 28* 948–60, 1967

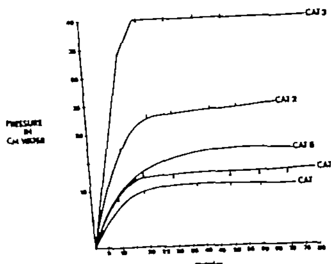


Fig 4 In 5 cats studied, middle ear pressure rose to 12 to 40 cm of water. After 25 min middle ear pressure remained constant.

Matz, G. J. et al. (ibid.), 1967

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1969 Baker 1969 Doult, 1965) In this way the transient cardiovascular and respiratory depression of the barbiturate is avoided.

4 Points of General Interest and Possibilities of Protected Sleep

It is of interest to note that the success of the technique would seem to depend on the *administration of a sufficient dose from the start* adapted of course to age general condition and degree of nervousness of each patient. With this narco-neuroleptanalgesia we can obtain a surgical field with minimal bleeding, minimal oozing and with good conditions for dissecting in fields of E.N T surgery (particularly ear surgery) neurosurgery ophthalmic surgery and maxillo-facial surgery

Our observations have shown. (*a*) a microsurgical field with minimal bleeding and minimal oozing (functional ear surgery microscopic neurosurgery) (*b*) a low cerebro-spinal fluid pressure (*c*) a decreased brain volume (*d*) a low intraocular tension (ophthalmology) (*e*) a very gradual and calm recovery with little or no postoperative cerebral oedema, lesser postoperative hyperthermia, and no postoperative hemorrhage (*f*) in neurosurgery an immediate control of the respiratory centre is possible throughout the operation.

B Practical Application of the Technique

1 Functional Ear Surgery

A too-often disregarded problem in otological surgery is the type of anaesthesia used. Among the many works published on ear surgery those discussing or even mentioning this problem are extremely rare. It is nevertheless certain that anaesthesia in itself plays a role in the outcome of surgery be it simply local anaesthesia, loco-regional anaesthesia with or without premedication, or general anaesthesia with or without controlled respiration. The patient is brought temporarily into an abnormal and artificial physiological state from which the physiological cochlear equilibrium cannot avoid influence. Most often, however these considerations are not borne in mind, generally because of lack of interest on the part of the surgeon.

I believe that in the surgery which interests us here, we need to ensure not only good anaesthesia in which the reactivity of the autonomic nervous system is markedly reduced but also that the cessation of anaesthesia at the end of the operation does not provoke a sudden alteration in the equilibrium obtained during the period of an esthesia, especially that affecting the labyrinthine circulation (Marquet, 1970)

When performing middle ear surgery under inhalation anaesthesia administered by orotracheal intubation, the surgeon often observes a bulging of the tympanic membrane, of the fascia or veingraft or of the homograft. The bulging increases until it is so pronounced that gas escapes from under the edge of the graft. The eardrum or the graft is seen to collapse, but soon the bulging will reappear Quite obviously a positive pressure is generated in the middle ear This phenomenon is caused by nitrous oxide The essential factor is the difference between the partition coefficients of nitrous oxide and nitrogen (Thomson, Terkildsen & Arnfred, 1965 Rasmussen, 1967 Waun, Sweiter & Hamilton 1967 Matz, Rattenborg & Holaday 1967 Foldes, Kapes & Ship, 1965)

The solubility in blood of nitrous oxide is thirty times larger than for nitrogen and thirty times more nitrous oxide than nitrogen can be carried at the same partial pressure As a consequence of this difference the blood arriving at the middle ear can discharge a large quantity of nitrous oxide into the cavity and

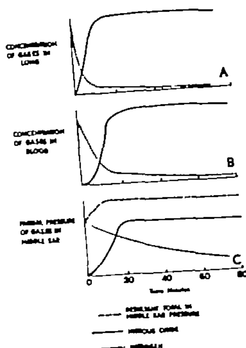


Fig. 3 (*A*) show rapid increase of pulmonary nitrous oxide with a concomitant decrease in nitrogen concentration. This is associated (*B*) with similar changes in blood. This results in rapid increase of nitrous oxide partial pressure in the middle ear associated with gradual decrease in the partial pressure of nitrogen, resulting in an increase in middle ear pressure (*C*).

Matz, G. J. Rattenborg, C. G. & Holaday D. A. Section of Otolaryngology and of Anesthesiology University of Chicago School of Medicine, Chicago. Effects of nitrous oxide on middle ear pressure. *Anesthesiology 28* 948–60, 1967

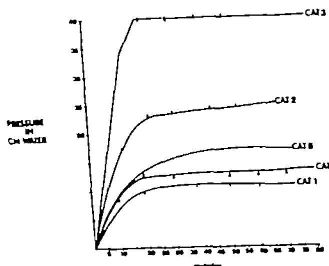


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Fig. 6. Bed ready to receive patient still in the state of "Protected Sleep

Once the patient has been put back to bed in a correct attitude, the postoperative control becomes very simple. It consists only of checking pulse rate, respiration, temperature and blood pressure. Putting the patient back to bed is done with special care and attention because of the prevailing general muscle relaxation, vasoplegia, low venous pressure, relative arterial hypotension and absence of muscle activity (Fig. 6).

Any point of pressure upon the body must be protected. After every period of 2 hours it is recommended that the patient be slightly moved in order to expose other points of the body to the pressure of the bed surface. One should pay special attention to the coccyx, the pericoccygeal region and the skin on the occipital part of the head (Abel, 1964). Elongation of the plexus brachialis or compression of the ulnar nerve must of course be avoided (Jackson & Keats, 1965 Boulton & Cole, 1967) For all this the patient receives a thick and soft pillow underneath the head and shoulders (the head is turned a little sideward with the chin pointing upward). The arms are folded and a small pillow is put under each elbow The hands are put on the thorax or on the abdomen. A large soft cushion is put underneath the lower extremities. The heels hang free. The blankets are laid over the foot of the bed. In order to avoid also that the ankle joint be brought into extension, the sheets are not fixed underneath the foot of the mattress. One also makes sure that folds neither of pyjamas nor of bed sheets exert any pressure on the pericoccygeal region or on the thighs. During the first 24 hours, blocks (height, 15 to 20 cm) are put under the foot of the bed in order to prevent venous stasis in the lower limbs. (This is an interesting help to any postoperative patient.)

Both corneal and plantar reflexes are nearly always present at the end of the operation. After 1 to 2 hours, the swallowing reflex returns. The oral cannula or tube, however is still tolerated at this time. After a period of 4 to 6 hours the patient can be stimulated and be spoken to He goes to sleep each time, however During the entire first day and night he is only allowed to suck ice cubes (dry mouth).

4 *Further course*

Very rarely postoperative analgesics are needed. If so Methampyrone (Novalgin®) is given by i.m. injection. The next day patient drinks and light food is allowed. In fact, the patients should be encouraged to drink quite a lot, this improves diuresis and excretion of the admin-

— The surgeon starts operation with a local infiltration of about 1 ml of 1 or 2% Prilocaine, Lignocaine or Carbocaine with 0 1 mg Epinephrine. Usually this is done at about 8 mm from the tympanic membrane

The purposes of this infiltration with local anaesthetics are (1) additional local anaesthesia, (2) additional prevention of even minimal bleeding, and (3) to perform an easy dissection of the skin from the external auditory canal. If patient reacts upon local infiltration 5 to 10 mg Diazepam is injected slowly (flooding of vein should follow)

(*b*) During the surgical intervention a small dose of the P N L mixture is given according to routine after about 90 min of operation time or in the event of any reaction of the patient. An additional dose of Diazepam (5 to 15 mg) may also be indicated.

Observations

— We observe a decrease of arterial blood pressure (systolic as well as diastolic) with an average systolic value of 80 to 100 mmHg in normotensive patients

— The venous pressure is low as can be observed clinically in the veins of the extremities and at the jugular vein.

— *Respiration is sufficiently deep and calm*, and there is no bronchial hypersecretion.

— Peripheral circulation is adequate as judged from both the extremities and the microscopic operation field.

— Upon reposition or homograft of the tympanic membrane, the latter does not balloon

Remarks.

(*a*) Children and young adults should receive higher doses of the P N L. mixture than might be expected The doses required depend more on the metabolism of the organism than on the body weight. However multi-fractionated administration of the drugs is in any case the method of choice

(*b*) After the large induction dose of the narco-neuroleptic mixture the autonomic nervous blockade of the sympathic as well as of the vagal system is illustrated, in most patients, by the tendency of the heart rhythm towards tachycardia. After a variable period of time, the physiological vagal preponderance will gradually exert its influence again and slow down the heart to a frequency of 70 to 100 per minute

(*c*) Light hypothermia is a usual consequence of this kind of anaesthesia (±35 to 35 5 C).

(*d*) Spots of intense intracutaneous vasoplegia sometimes appear following the intravenous injection of the narco-neuroleptic mixture. They disappear spontaneously after 20 to 45 min

(*e*) In case of insufficient bloodlessness at the site of microsurgery i v injection of an additional dose of Levomepromazine (25 to 50 mg) and of 0 6 to 0 9 mg. of Hydergin (Goodman & Gilman 1965) in order to reinforce the alpha adrenergic blockade If this is still insufficient, give once more the mixture of Pethidine 100 to 200 mg, Nalorphine 2.5 to 5 mg, Levomepromazine 25 to 50 mg and Diazepam 5 to 10 mg in order to deepen the anaesthesia. In this manner peripheral autonomic nervous reactions which affect the vascular tone may be prevented more effectively If this procedure is also not helpful in preventing bleeding at the operation site, a slow i v injection of one or two vials of Epsilon Amino-Caproic Acid (Capramol® vial containing 2.5 g) (Berger & Pinson, 1964) is recommended. This is on the basis of the hypothesis that in some patients local fibrinolysis may occur at the site of protracted microsurgery because of liberation of tissue kinases.

3 *Immediate postoperative measures*

During this period not the least anaesthesiologic manipulation is required The patient is put to bed again, while still in "Protected Sleep" At this time it is sometimes useful to inject a dose of 5 to 10 mg of *N* Allyl Normorphine intravenously In patients with a cardiovascular or pulmonary handicap this can be supplemented by an intravenous or intramuscular preparation of a Theophylline derivate.



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Anaesthetic management

Intramuscular premedication. Pethidine, Levomepromazine and Diazepam. Dose according to clinical state and age of each patient. For example normal adult. Prethidine 100 mg, Levomepromazine 25–50 mg, Diazepam 10 mg.

Induction of anaesthesia: a normal initial C.V dose of Thiopentone 5% (300 to 600 mg for an adult) or an induction dose of Diazepam (20 to 40 mg for an adult) is followed by a more or less large dose of the Pethidine Nalorphine-Levomepromazine mixture according to clinical state and age (from 1/8 to the whole of the mixture) Relative proportions. Pethidine 300 to 400 mg Nalorphine: 7.5 to 10 mg Levomepromazine 75 to 100 mg.

A single dose of suxamethonium 25 to 50 mg to facilitate tracheal intubation is added. The vein should be flooded after the administration of the P.N.L. mixture (see further in Complications)

For maintenance nitrous oxide, oxygen, small amounts of Halothane and additional doses of the mentioned mixture are given. Uni-directional gas flow with the Magill attachment and an expiratory Ruben valve or a similar low resistance valve is provided. Fresh gas flow of 5 to 7 l min, which is largely sufficient to prevent rebreathing (Norman, Adams & Sykes, 1968 Mapleson, 1954). If the neurosurgeon wishes to do so, he may use without fear a local anaesthetic on the skull, even with adrenalin. The induced autonomic nervous blockade is sufficient to reduce reflex sympatho-adrenal activity

At the slightest reaction of the patient (bucking on the endotracheal tube or a slight movement) a certain amount of the P.N.L mixture will be administered without hesitation.

The same applies to a rise in the arterial pressure or an acceleration of the respiration. The quantity of halothane vaporized per hour is minimal (4 to 8 ml) and will depend on essentially two factors. the arterial pressure (kept between 70 and 100 mm of mercury systolic) and no or minimal depression of the spontaneous respiration. If Halothane is administered in higher concentrations the opposite of the desired could be the result (MacDowall, Barker & Jennett, 1966 Jennett et al. 1967 Jennett, Barker Fitch & MacDowall, 1969)

When the arterial systolic pressure reaches 70 mmHg, or if respiration is even but slightly depressed, Halothane administration will be momentarily interrupted.

At the termination of surgery the extubation and the introduction of an oral cannula should be performed as gently as possible without coughing or other tracheal or laryngeal reactions (rise in venous pressure and rise in C.S.F pressure) (Freund, 1968)

We do not hesitate to administer just prior to this manipulation 4 to 5 ml of Thiopentone 5% 5 to 10 mg Diazepam, or even a certain amount of the P.N L. mixture. The endotracheal tube could also be left in situ when desired. In our opinion *the method of the early awakening of these patients on the operation table is to be completely avoided.* The complete recovery of normal reflexes and of normal consciousness should be postponed for several hours and happen as gently and gradually as possible. Because of this prolonged state of Protected Sleep (Figs. 7 8) pressure points should be protected (see further in postoperative management in ear-surgery).

During the surgical procedure, as well as in the postoperative period intravenous fluid therapy and blood transfusion (rarely necessary) should be restricted to a minimum. This is in order to avoid venous pressure rise, C.S F pressure rise, cerebral oedema and postoperative hemorrhage. The brain is most sensitive to water intoxication (Rosomoff, 1968).

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Vertigo is rarely observed after this type of anaesthesia. Obviously the kind of surgical intervention plays a role too. Nausea or vomiting occur very rarely if so Metoclopramide (Primperan®) (Tornetta, 1969 Brassier 1966) is the drug we use. On the third postoperative day the patient can resume his normal activities Postoperative microscopic ear-dressing is done but in case of transplant or homograft of membrana tympani, dressing is only made on the fifth day

The patient can feel a certain buttock stiffness. It disappears with normal muscle activity It is caused mainly by the intramuscular injections.

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2. Neurosurgery

In the great majority of neurosurgical departments all over the world, pulmonary hyperventilation for neurosurgery remains the technique of choice (Allen & Morris, 1962 Schettini et al 1967 Rheault & Lepage 1967 Gilbert, Brindle & Galindo 1968 Douglas et al 1969 Krumperman, 1969)

This technique is considered to improve operating conditions, to reduce the amount of anaesthetic drugs required for adequate relaxation, to reduce the volume of the brain and the intracranial pressure and to bring about a rapid recovery of consciousness (Pierce, 1964 Gordon 1968) Under usual clinical conditions, however an increase in ventilation decreases arterial pCO_2 and hence cerebral blood flow is lowered This diminished blood flow could completely negate or even reverse the desirable effects produced by increased pulmonary ventilation Most outstanding studies challenge the assumption that hyperventilation because of cerebral vasoconstriction results in reduction of intracranial pressure and brain volume. It has been found that during hyperventilation a decrease in intracranial blood volume was accompanied by a compensatory increase in cerebrospinal fluid volume (Rosomoff, 1963) Some interesting studies have also shown that cardiac output and oxygen uptake are reduced in patients on controlled respiration (Malcolm-Smith Grenvik & Westerholm 1968)

Another point of some interest is the hyperoxygenation which accompanies controlled ventilation. Some investigations have shown that hyperoxygenation by itself produces peripheral vasoconstriction and decreases circulation. Autonomic nervous rather than local metabolic effects are believed to cause these changes (Bergofsky & Bertun, 1966 Bauer & Gravenstein, 1966).

Since much interest surrounds the role of brain pH in the regulation of cerebral blood flow experiments on this object have been performed. They demonstrated that during prolonged hyperventilation a metabolic acidosis develops in the cerebrospinal fluid, due to the accumulation of lactic acid (MacDowall & Harper 1968)

Moreover at a certain moment artificial ventilation must be stopped and after this a quite often stormy recovery period with the absolute necessity of intensive reanimatory care begins. A rebound" cerebral blood flow elevated above normal in the post hyperventilation period due to the cerebrospinal fluid acidosis is most likely to occur (MacDowall & Tarper 1968) Pulmonary and haemodynamic disturbances, postoperative hypoxemia, cerebral oedema, hyperthermia and focal hemorrhage are in fact not uncommon

We wonder whether this is not due in the main to improper operative anaesthetic management, where everything happens in subordination to and in consequence of the predominant concept the lighter the anaesthesia, the quicker the recovery the safer will be the technique

It might seem presumptuous to say so, but we are really convinced that the technique of Protected Sleep has something to offer (Delaruelle, 1968 1969 Delaruelle & Granieri, 1968, 1969)

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Another point of some interest is the hyperoxygenation which accompanies controlled ventilation. Some investigations have shown that hyperoxygenation by itself produces peripheral vasoconstriction and decreases circulation. Autonomic nervous rather than local metabolic effects are believed to cause these changes (Bergofsky & Bertun, 1966 Bauer & Gravenstein 1966)

Since much interest surrounds the role of brain pH in the regulation of cerebral blood flow experiments on this object have been performed. They demonstrated that during prolonged hyperventilation a metabolic acidosis develops in the cerebrospinal fluid, due to the accumulation of lactic acid (MacDowall & Harper 1968)

Moreover at a certain moment artificial ventilation must be stopped and after this a quite often stormy recovery period with the absolute necessity of intensive reanimatory care begins. A rebound" cerebral blood flow elevated above normal in the post hyperventilation period due to the cerebrospinal fluid acidosis is most likely to occur (MacDowall & Tarper 1968) Pulmonary and haemodynamic disturbances, postoperative hypoxemia, cerebral oedema, hyperthermia and focal hemorrhage are in fact not uncommon

We wond r whether this is not due in the main to improper operative anaesthetic management, where everything happens in subordination to and in consequence of the predominant concept the lighter the anaesthesia, the quicker the recovery the safer will be the technique"

It might seem presumptuous to say so, but we are really convinced that the technique of Protected Sleep has something to offer (Delaruelle 1968 1969 Delaruelle & Granieri, 1968 1969)

Camplin, 1968 Terry Daw & Michenfelder 1962 Uihlein, Terry Payne & Kirklin, 1962 Karlsberg & Adams, 1962 Michenfelder Terry Daw MacCarty & Uihlein, 1963 Rollason & Latham, 1963). For this purpose we use Trimethaphan (Rollason & Hough, 1960 Tough, 1950· Forgacs & Saabe, 1967 Robertson, 1959) at a concentration of 5 mg/ml. A test dose of 5 mg is injected intravenously and will be completed by intermittent, repeated doses af 5 to 15 mg with each injection. We prefer this to a continuous drip infusion. During this period we will check even more carefully the peripheral circulation (fingernails, toes), the pulse, the spontaneous respiration (depth and frequency) and of course the arterial tension. Venous pressure, E.C.G and E.E.G recording are of course very interesting but the pity is that in our country they are not always available At the same time 50% N_2O with 50% O_2 will be inhaled, without Halothane.

3 Ophthalmic Surgery

Intraocular pressure is influenced by the same factors as those controlling the volume of the intracranial contents and ophthalmic surgery imposes demands similar to those of neurological surgery (Adams, 1966) In ophthalmic surgery one is frequently confronted on the one hand with older patients, mostly in a poor general condition, or on the other hand with very young patients Neverth.less, the same basic technique can be applied, but the dose of the pharmacodynamic P N L. mixture should be adapted accordingly We can illustrate this with the following examples. In telegraphese·

Cataract or glaucoma surgery Patient 70 to 80 years Poor cardio-vascular and pulmonary conditions Premedication. Pethidine 25 to 50 mg. Levomepromazine 12.5 to 18 75 mg, Diazepam 5 to 5 mg. i.m. Together with it a suppository of a theophylline derivate (250 to 300 mg)

The P N L. mixture produces a pronounced miosis. Instillation of a Epinephrine–Cocaine solution is therefore obligatory in cataract surgery in order to obtain the desired mydriasis. This should be done 1 hour before the operation. Another alternative is a retrobulbar injection of a 4% Procaine solution just after the induction of the general anaesthesia. Anaesthesia. Thiopentone 5% (very slowly)· 300 to 400 mg, (or Diazepam. 10 to 15 mg) Pethidine. 30 to 50 mg; Levomepromazine: 12.5 to 18 75 mg· Nalorphine· 0.5 to 1,5 mg· Succinylcholine: 25 mg (Taylor Mulcahy & Nightingale, 1968). Dilution of the P.N.L. mixture and flooding of the vein with ± 20 ml of normal saline or glucose 5% solution (see further in Complications) Tracheal intubation nitrous oxide, oxygen, halothane. Spontaneous respiration. Ruben valve or similar alternative If necessary during surgery· additional doses of Thiopenton 5% each time 50 to 100 mg or Diazepam 5 to 10 mg, and when necessary a second small dose of the P.N.L. mixture. Gentle extubation, oral cannula.

Installation in bed with protection of pressure points by means of a soft cushion under the shoulders and occiput, a soft cushion under each elbow and a soft cushion under the calves, leaving the heels free. Gentle shifting of the patient every 2 hours in order to prevent any compression or decubitus-effects. Continue as long as the patient stays somnolent.

For ophthalmic surgery in the very young (most often strabismus surgery) we should, however prefer Promethazine before Levomepromazine under the age of 5 years (Eckenhoff, Helrich & Ralp, 1957) It is sufficiently hypnotic and protective but less hypotensive and it is to be preferred because of the lack of maturity and the greater lability of their autonomic nervous system. For this same reason did we not dare to use Diazepam in the very young. With this type of anaesthesia the well known oculo-cardiac reflex which may lead to circulatory crises (Kasper 1967) is no longer to be feared.

For example (again in telegraphese)·
Normal child, 3 years, strabismus surgery Pre

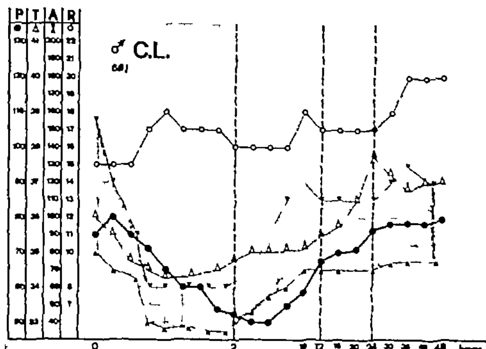


Fig 7 Representation of pulse (*P*), rectal temperature (*T*), systolic and diastolic arterial pressure (*A*) and respiratory rate (*R*) in a male patient, C. L., with a severe chronic bronchitis, operated for a right frontal tumour Duration of surgery 2 hours, 40 min. Parameters after induction of anaesthesia, after h, 12 h, 24 h, and 18 h.

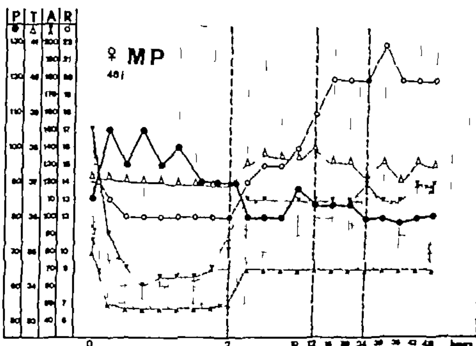


Fig 8 Representation of pulse (*P*), rectal temperature (*T*), systolic and diastolic arterial pressure (*A*) and respiratory rate (*R*) in a female patient, M P in coma for 3 days, operated for a right central cyst. Duration of surgery 3 hours, 15 min Parameters after induction of anaesthesia, after 2 h, 12 h, 24 h, and 48 h.

200 mg i m) is most often given in order to diminish further the effects of the central irritability due to the neurosurgical manipulations or neurological lesions

Intra cranial arterio-venous malformation

The same technique is employed in surgery for intracranial arterio-venous malformations. However during the more delicate phase of this vascular cerebral surgery a reinforced arterial hypotension, down to ±50 mmHg systolic, will *be realized. Thanks to the induced conditions* of sufficient gradient in the tissue blood flow and of moderate hypothermia this short reinforced hypotensive period will be quite well tolerated

It is certainly more controllable and less hard to realize than other techniques such as profound hypothermia, local circulatory arrest extracorporeal circulation or selective brain cooling (Rosomoff 1968 Gott, 1966 Deligne & David, 1966 Hoffmann & Merckx, 1966

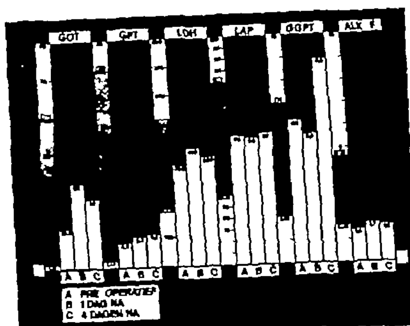


Fig. 9 Average levels of G O T G P T L D.H., L.A.P G.G T P and Alk.Phos the day before, the first day after, and 4 days after anaesthesia in series of patients for microsurgery in the ear

most) but all of them remain within the normal limits of the technique used (Figs. 9 10)

(c) *Electrocardiographic recordings* have *sometimes shown alterations of the ST-segment, the T wave as well as axis-deviations.* These transitory alterations were also seen in younger patients. Indeed, such patients receive relatively higher doses. Consequently their basal metabolism is being altered more *intensively* The E.C.G alterations observed may be the consequence of a generally decreased cell metabolism or cell membrane alterations, expressed at the level of the heart muscle. They may also be the expression of a central neuro-humoral mechanism (Granieri & van Bogaert, 1968 Tsunoda 1964) or of an interference with myocardial biochemistry or contractility (e.g. potassium *extra-intracellular imbalance*) (*List*, 1968 *Laborit*, 1959).

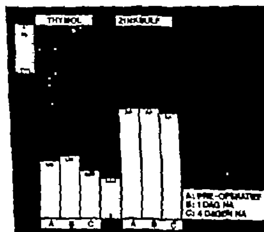


Fig 10 A erage levels of Thymol and Zinc Sulf., the day before, the first day after and 4 days after anaesthesia in series of patients for microsurgery in the ear

Similar E.C.G alterations were found with different phenothiazines (Leak & Carroll, 1967 Goodman & Gilman, 1965). A real myocardial ischemia or infarction is not likely to produce the E.C.G alteration observed after the use of the P.N.L. mixture. The clinical state of the patients, the absence of S G.O T elevation and the *transitory nature of the E.C.G. findings* support this view (*Kelley Cambell & Brandt*, 1967)

Several cardiac patients, either with congenital malformations of the heart or with a history of cardiac infarction have been anaesthetized and have been operated on successfully The same can be said of several patients with other *forms of circulatory insufficiency* Because of

medication Pethidine 25 mg, Promethazine 25 to 35 mg Atropine Sulf 0 3 to 0 5 mg. Anaesthesia. Induction by means of an anaesthetic mask with nitrous oxide oxygen and Halothane Intravenous administration of 100 to 150 mg of Thiopentone 5% followed by Pethidine ± 25 mg, Promethazine 25 to 40 mg and ± 20 mg of Succinylcholine. Gentle intubation with an appropiate size of tube. Nitrous oxide oxygen Halothane. Spontaneous respiration Gentle extubation of the still sleeping child and installation in bed with an oral cannula in situ

Remarks.

For operative procedures such as enucleation without or with an implant or for dacryo-cystorhinostomy the same reinforced arterial hypotension with Trimetaphan as described for arteriovenous malformations in neuro-surgery will be realized

Quite frequently glaucoma patients received potent diuretic agents before coming to the hospital. These result in a potassium depletion. Anaesthesia and neurolepsia also provoke a certain degree of hypokalemia (Laborit, 1959 1959 Du Callar 1961) Thus, potassium should be administered to these patients before and after operative treatment.

4 Maxillo-facial and E.N T Surgery

The same technique as for neurosurgery and ophthalmic surgery is suited to maxillofacial and E.N T surgery The only exception being functional ear surgery (Delaruelle & Marquet, 1966)

C Control Investigations

All the control investigations were performed in the ear surgery series. Indeed only here is the parenteral route alone used for anaesthesia. There is no possible interference from other anaesthesic agents, gases vapours or even of oxygen administration

(a) During the first hour following surgery the most critical period" an Astrup capillary blood control has been made in a series of patients The results show a very mild degree of purely respiratory acidosis The average values are for the pCO_2 46 mmHg and for the pH 7 38 The two extreme values obtained are respectively 40 mmHg and 51 mmHg (this in a chronic bronchitis, with emphysema) and 7 46 and 7 32 for the pH

These results are similar to those found in non anaesthetized subjects, at night during a period of so-called deep sleep (Comroe, 1966) Metabolic acidosis is not present. The average values are for the Base Excess − 1 mEq/l, and for the Standard Bicarbonate, 23 8 mEq/l The two extreme values obtained are, respectively − 3 mEq/l and + 5 mEq/l for the Base Excess, and 21 mEq/l and 28 mEq/l for the Standard Bicarbonate.

Since metabolic acidosis accompanies cellular hypoxia we might conclude that the cellular perfusion and oxygenation stayed within normal limits (Schmier 1969)

(b) Liver function tests were performed in the laboratory of the Bunge Institute (Mr Swinnen, biologist) Levels of some cell and secretion enzymes were assayed before and after anaesthesia for ear surgery in addition to serum bilirubin and two flocculation tests. G O T (Reitman & Fränkel), LDH (Cabaud & Wroblewski) G G T P (Swinnen), L A P (Weber) Alkaline phosphatase (Bodanski) Thymol (MacLagan with buffer according to Reinholds) Zinc sulfate (Kunkel) and Bilirubin (Weber and Schalm)

The results show the expected influence on the secretory function of the liver but no hepatoxic influence The activities of serum enzymes increase in a variable manner (GGTP

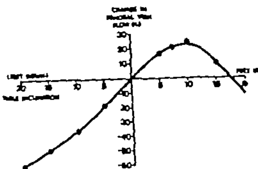


Fig 11 Experiments in greyhounds have been conducted to assess the effect on venous return of varying degrees of operating-table tilt. The mechano-haemodynamic response of the enous system of the greyhound hind limb is almost identical with that in man. Changes in the mean venous-flow rates were measured by electromagnetic *flow* probes placed around the exposed femoral eins. The results indicate that for optimal promotion of venous return the bed should be inclined at 10 to the horizontal.

Roberts, V C., Department of Biomedical Engineering King College Hospital Medical School, London *The Lancet 7653* 948, May 1970.

introduce most often a catheter in a large vein and flush after each injection.

Twenty-six times we encountered a clinical thrombophlebitis in the legs and eleven times a mild but clinically unmistakable lung embolism All these patients were discharged in good health. Nine of these lung embolisms occurred in female patients. We do not know if these patients were taking contraceptive pills. Today when we encounter a patient with manifest varicosities or with a history of venous insufficiency in the lower limbs, a prophylactic treatment with a Hydergin prepartion (PH 203 Sandoz) is administered by i.m injection, for 3 to 5 days postoperatively The use of a 10 head down position during the first 24 hours might also prevent to some extent these vascular complications (Fig. 11).

Seven times we encountered a transient ulnar nerve palsy and once a branchial plexus injury The first case of transient ulnar nerve palsy was a young man who afterwards underwent a careful neurological examination and a electromyographic study A very superficial ulnar sulcus at the elbow could be demonstrated. During surgery the patient's arm had been lying on an ordinary lateral armboard. With physiotherapy and vitamin B therapy the nerve recovered in 6 months.

A more embarrassing complication was that of a 65-year-old male patient, whose arm had also been lying on a lateral armboard and who developed an ulnar nerve and a medial nerve injury after surgery Postoperative examinations showed an advanced degree of cervical ankylosing spondylitis.

Since these occurrences, we no longer use an arm rest but, whenever possible, cross the arms on the abdomen and protect shoulders and the elbows with soft cushions.

Postoperative mental disturbance (Blundell, 1967) that could be attributed to a senile or presenile dementia occurred in 19 patients. They were all over the age of 60 Two cases were operations for trigeminal neuralgia and the rest of them were all intraocular surgeries. Most bad risk and hyperanxious patients belong to the same series. Today whenever we can find in the history of these older patients the slightest hint of manifest malnutrition or of somewhat presenile or senile psychotic behaviour we hydrate the patient intravenously and at the same time administer a certain amount of potassium. An intramuscular purified liver extract (Neo-Hepatex® Evans) is also given for 3 to 4 days. This may seem unimportant, but we do this with the idea of fortifying these older patients. It goes without saying that the dose of the P.N.L. mixture should be most carefully adapted in these cases.

the prevailing complete peripheral vasodilatation and the low venous pressure the relative arterial hypotension is less dangerous in this method. The *normal blood flow* gradient remains here. The myocardial work load function also seems diminished but decreased peripheral vascular resistance permits the heart to maintain a greater forward output than would be possible if aortic pressure remained high (Siegel, 1969)

This does not occur in methods of artificial ventilation as used in light anaesthesia, where a certain degree of vasoconstriction and a higher venous presure are always found. This way both tissue perfusion and oxygenation are impaired in advance. Such a condition is, as already stated, potentially dangerous.

In Protected Sleep" *haemodynamic* pressure changes always occur very gradually There are never any sudden transitions. This is also true at the end of the surgical intervention.

D Complications

Having applied the technique for 9 years we venture to say that this technique is safe. We also venture to assume that no death occurred as a direct consequence of the anaesthetic technique in itself.

Out of a total of 5 628 8 patients died on the operative day and 46 others died during hospitalisation. 37 of these 54 in the neuro-surgical series due to preexisting irreversible neurological damage. One death (in 1963) was that of a glaucoma *female patient of 72 years who died 6 days* postoperatively Preoperatively she had received for several days, large doses of a potent diuretic (Diamox®) After surgery she had shown mental disturbance and a starvation syndrome. We think that the anaesthesia and the neurolepsia precipitated the already existing potassium imbalance and caused irreversible damage One other death was that of a pale, fatty 3-year-old boy operated on for strabismus. The *following morning the boy presented a shock" syndrome and could not be reanimated.* We are unable to explain the exact reason for this lethal complication Autopsy seemed to reveal an impaired desintoxication function Pethidine, Nalorphine and Promethazine could be extracted from liver and kidney tissue. The boy had also *received* Halothane and during the post operative night a suppository *of* Aspirin–Phenacetin–Codein. The *other deaths* were due to various causes but had one *factor in common* All of them were bad risk" patients

Decubitus lesions were encountered in 76 patients. Fortunately only three of them were severe. The great majority of the cases occurred during the first years of application of the technique. At that time we had not yet fully realized that decubitus and local compression are always possible whenever a patient remains *immobile for several hours. Especially after* hypotensive anaesthesia and when lying on the hard, thick rubber pads which our hospitals use to protect their mattresses. Now we pay special attention to the protection of pressure points (see chapter on immediate post-operative measures) and recommend the nursing staff to move the patient slightly every 2 hours.

The lesions encountered were mostly *local*ised in the pericoccygeal area. *Treatment consisted of an intravenous infusion of* 500 ml Rheomacrodex® with 3 to 4 ml heparin. A four times daily application of Butazolidine® ointment completes this therapy

Local induration of a vein after i.v injection of the mixture of drugs was seen in 208 patients.

Dilution of the *Pethidine and the rest of the mixture in a sufficient amount of normal saline or glucose* 5% makes this minor but undesirable side effect much less frequent. Today we

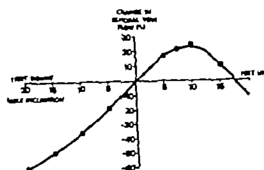


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on de Sommeil Protégé Nous estimons cet aspect particulier est très important. a nous permet d'obtenir
une pCO_2 constante
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une pression veineuse basse et stable.
out cela aussi bien pendant la période post opératoire immédiate que pendant la période opératoire et cela sans transitions brutales. Cet at anesthésique en outre est caractérisé par ne hypotension artérielle, une très bonne cir ulation périphérique et capillaire, ainsi que par une légère hypothermie.

Nous obtenons ainsi un champ opératoire aussi exsangue que possible, ce qui constitue un atout majeur pour des disciplines chirurgicales où la possibilité d'hémostase locale est très limitée la chirurgie O.R.L. (surtout la microchirurgie de l'oreille), la neurochirurgie, la chirurgie ophthalmologique et la chirurgie maxillo-faciale. La période post-opératoire est caractérisée par un réveil progressif et non agité.

La méthode est décrite du point de vue clinique et pratique. Des considérations théoriques ne sont mentionnées que pour expliquer un point particulier de la technique.

Zusammenfassung

Mit der beschriebenen Narkose-Methode wird ein Zustand erreicht, der charakterisiert ist durch eine Analgesie sowie eine tiefe und anhaltende Neurolepsie. Die neurovegetativen Reaktionen sind erheblich reduziert.

Es gibt keine Anzeichen von Atemdepression. Die Atmung ist der eines physiologischen Tiefschlafes vergleichbar daher die Bezeichung überwachter Schlaf" (sommeil protégé).

Wir sind der Meinung, dass dieser besondere Aspekt von grosser Bedeutung ist.
Dies ermöglicht.
— einen konstanten CO_2 Partialdruck
— einen vasokonstriktionsfreien zentralen und peripheren Kreislauf
— einen niedrigen und stabilen Venendruck
und zwar nicht nur während des Eingriffs, sondern auch unmittelbar nach der Operation, ohne scharfe Transitionen zu zeigen. Dieser Narkosezustand ist ausserdem charakterisiert durch eine arterielle Hypotonie, eine sehr gute Peripherie und Kapillardurchblutung, sowie durch eine leichte Hypothermie.

Auf diese Weise verfügen wir über ein möglichst blutleeres Operationsfeld, was einen bedeutenden Vorteil für die chirurgischen Disziplinen darstellt, in den Fällen, wo die lokalen Hämostasemöglichkeiten sehr beschränkt sind. O.R.L.-Chirurgie (vor allem die mikrochirurgischen Eingriffe am Ohr), die Neurochirurgie, die Augenchirurgie und die Oberkiefer-Gesichtschirurgie. Die postoperative Phase ist charakterisiert durch ein allmähliches, ruhiges Erwachen.

Die Methode wird aus klinischer und praktischer Sicht beschrieben. Theoretische Betrachtungen dienen nur zur Erklärung technischer Einzelheiten.

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E Patients' Statistics

A total number of 5 628 operations were performed under the described type of anaesthesia, from 1960 until Juli 1969

(1) *Microscopic ear surgery* (age range of patients, 5 to 74 years)

Stapes surgery	1 360	
Reconstructive surgery of the ear (with or without homograft)	1 244	
Surgery of the facial nerve (either through os petrosum or by external approach	45	
Congenital ear atresia	18	2 677

(2) *Neurosurgery* (age range of patients, 18 months to 81 years)
The author used the Protected Sleep" technique of anaesthesia in 765 cases out of a total of 1 914 neurosurgical operations which were performed by Dr P Selosse from 1961 until 1969 In the other cases anaesthesia was administered by a colleague Dr A. Soetens

Cerebral tumours (or similar types of intervention)	272	
Cerebral arterio-venous malformations	12	
Cranial traumata (among others extra- and subdural hematomata)	98	
Operations on the cervical and lower spine (among others, nucleus pulposus surgery)	359	
Miscellaneous (among others trigeminal neuralgia hydrocephaly focal epilepsy cerebral biopsy)	24	765

(3) *Ophthalmic surgery* (age range of patients, 2 to 94 years)

Cataracts	671	
Glaucomas	352	
Corneal grafts	15	
Enucleations (with or without implant)	33	
Retinal detachments	69	
Dacryo-cystorhinostomies	54	
Eyelid plastics	27	
Strabismus	672	
Miscellaneous	16	1 909

(4) *Maxillo-facial and E.N T surgery* (age range of patients, 12 to 78 years)

Maxillar and mandibular tumours	55	
Paradental cysts	30	
Larynx surgery	76	
Thyroid surgery	82	
Parotid surgery	37	
Neck surgery (mostly for tumours)	21	
Vocal cord surgery	3	
Nose and sinus surgery	83	387
	Total	5 628

Résume

La méthode d anesthésie décrite provoque un état caractérisé par une analgésie et une neurolepsie profonde et résiduelle Les réactions neurovégétatives sont sensiblement réduites.

Il n'y a pas de signes de dépression respiratoire. La respiration est *tout à fait comparable* à celle observée chez un sujet en sommeil physiologique au stade profond, d ou la dénomi

sation de « Sommeil Protégé ». Nous estimons que cet aspect particulier est très important. Cela nous permet d'obtenir
— une pCO_2 constante
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— une pression veineuse basse et stable.

Tout cela aussi bien pendant la période post-opératoire immédiate que pendant la période opératoire et cela sans transitions brutales. Cet état anesthésique en outre est caractérisé par une hypotension artérielle, une très bonne circulation périphérique et capillaire, ainsi que par une légère hypothermie.

Nous obtenons ainsi un champ opératoire aussi exsangue que possible, ce qui constitue un atout majeur pour des disciplines chirurgicales où la possibilité d'hémostase locale est très limitée : la chirurgie O.R.L. (surtout la microchirurgie de l'oreille), la neurochirurgie, la chirurgie ophthalmologique et la chirurgie maxillo-faciale. La période post-opératoire est caractérisée par un réveil progressif et non agité.

La méthode est décrite du point de vue clinique et pratique. Des considérations théoriques ne sont mentionnées que pour expliquer un point particulier de la technique.

Zusammenfassung

Mit der beschriebenen Narkose-Methode wird ein Zustand erreicht, der charakterisiert ist durch eine Analgesie sowie eine tiefe und anhaltende Neurolepsie. Die neurovegetativen Reaktionen sind erheblich reduziert.

Es gibt keine Anzeichen von Atemdepression. Die Atmung ist der eines physiologischen Tiefschlafes vergleichbar: daher die Bezeichnung „überwachter Schlaf" (sommeil protégé).

Wir sind der Meinung, dass dieser besondere Aspekt von grosser Bedeutung ist.
Dies ermöglicht:
— einen konstanten CO_2 Partialdruck
— einen *vasokonstriktionsfreien zentralen und peripheren Kreislauf*
— einen *niedrigen und stabilen* Venendruck

und zwar nicht nur während des Eingriffs, sondern auch unmittelbar nach der Operation, ohne scharfe Transitionen zu zeigen. Dieser Narkosezustand ist ausserdem charakterisiert durch eine arterielle Hypotonie, eine sehr gute Peripherie- und Kapillardurchblutung, sowie durch eine leichte Hypothermie.

Auf diese Weise verfügen wir über ein möglichst blutleeres Operationsfeld, was einen bedeutenden Vorteil für die chirurgischen Disziplinen darstellt, in den Fällen, wo die lokalen Hämostasemöglichkeiten sehr beschränkt sind. O.R.L.-Chirurgie (vor allem die mikrochirurgischen Eingriffe am Ohr), die Neurochirurgie, die Augenchirurgie und die Oberkiefer-Gesichtschirurgie. Die postoperative Phase ist charakterisiert durch ein allmähliches, ruhiges Erwachen.

Die Methode wird aus klinischer und praktischer Sicht beschrieben. Theoretische Betrachtungen dienen *nur zur Erklärung technischer* Einzelheiten.

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E Patients' Statistics

A total number of 5 628 operations were performed under the described type of anaesthesia, from 1960 until Juli 1969

(1) *Microscopic ear surgery* (age range of patients, 5 to 74 years)

Stapes surgery	1 360	
Reconstructive surgery of the ear (with or without homograft)	1 244	
Surgery of the facial nerve (either through os petrosum or by external approach	45	
Congenital ear atresia	18	2 677

(2) *Neurosurgery* (age range of patients, 18 months to 81 years)
The author used the Protected Sleep" technique of anaesthesia in 765 cases out of a total of 1 914 neurosurgical operations which were performed by Dr P Selosse from 1961 until 1969 In the other cases anaesthesia was administered by a colleague, Dr A. Soetens.

Cerebral tumours (or similar types of intervention)	272	
Cerebral arterio-venous malformations	12	
Cranial traumata (among others extra- and subdural hematomata)	98	
Operations on the cervical and lower spine (among others, nucleus pulposus surgery)	359	
Miscellaneous (among others, trigeminal neuralgia hydrocephaly focal epilepsy cerebral biopsy)	24	765

(3) *Ophthalmic surgery* (age range of patients, 2 to 94 years)

Cataracts	671	
Glaucomas	*352*	
Corneal grafts	15	
Enucleations (with or without implant)	33	
Retinal detachments	69	
Dacryo-cystorhinostomies	54	
Eyelid plasties	27	
Strabismus	672	
Miscellaneous	16	1 909

(4) *Maxillo-facial and E.N T surgery* (age range of patients, 12 to 78 years)

Maxillar and mandibular tumours	55	
Paradental cysts	30	
Larynx surgery	76	
Thyroid surgery	82	
Parotid surgery	37	
Neck surgery (mostly for tumours)	21	
Vocal cord surgery	3	
Nose and sinus surgery	83	387
	Total.	5 678

Résumé

La méthode d anesthésie décrite provoque un état caractérisé par une analgésie et une neurolepsie profonde et résiduelle. Les réactions neurovégétatives sont sensiblement réduites.

Il n y a pas de signes de dépression respiratoire La respiration est tout à fait comparable à *celle observée chez un sujet en sommeil* physiologique au stade profond, d'ou la dénomi

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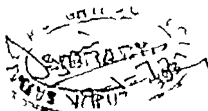
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BY

A. BRUNER and T W NORRIS[2]

Lovelace Foundation and Lovelace Clinic
Albuquerque, New Mexico, USA

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CONTENTS

I Introduction

Hearing loss, ataxia and complaints of vertigo are commonly encountered in old age. Ostensibly these disturbing symptoms, which do not necessarily appear concurrently may be interpreted as suggesting a degenerative process affecting the entire labyrinth. Although age-related cell loss has been demonstrated in the cochlea, corresponding changes within the vestibular portion have not been documented (Weiss, 1959)

The investigation of vestibular function and aging has been undertaken in a rather small number of studies. The diversity of methods employed in these studies impairs one's understanding of what changes, if any may be normally expected as a function of age. For vestibular nystagmus, the most frequently reported age effect has been hypo-reactivity (Arslan, 1957 Brookler & Pulec, 1970 Camarada & Lumia, 1959 Haas, 1964 Minnigerode, Grohmann & Vontin, 1967 Okano, 1938 Rossberg, 1964 Zelenka & Slaninova, 1964). A smaller number of reports have indicated heightened reactivity with age (Chladek, 1966 Guedry 1950 Kotyza, 1939) or no change (Bourhère, 1948 Forgacs, 1957 Fregly & Graybiel, 1970) A review and critique of this literature will follow the presentation of our own results on age and caloric nystagmus, and an attempt will be made to reconcile the various findings into a coherent picture.

II Material and Method

Subjects

The subjects were 293 patients selected from the total sample of 514 patients referred for hearing and vestibular testing primarily due to complaints of dizziness (Table 1). Every attempt was made to exclude subjects demonstrating positive clinical evidence that might contribute to abnormal vestibular function. The appearance of spontaneous or positional nystagmus in the absence of other clinical signs was not considered sufficient basis for exclusion Selection criteria excluded subjects having

1 Positive clinical evidence of pathology (e g Ménière's disease) or injury potentially associated with vestibular dysfunction such as *skull fracture cervical vertigo*, multiple sclerosis, suspected *tumors*, *etc*.

2. Clinically significant unilateral weakness (30–70% asymmetry or greater) based upon the caloric test;

3 *Hearing loss exceeding that of 95% of* subjects of comparable ages surveyed in the U.S. National Health Survey (Glorig & Roberts, 1965)

4 Incomplete data.

Caloric tests

Caloric irrigations of 250 *ml over* 30 sec were performed in the horizontal *supine* position with the head inclined 30 degrees. *Water* temperatures at the syringe tip were 31.5 C (cold) and 44 C (warm) controlled by Labline no. 3057 regulators. *The irrigation* sequence was right ear cold (RC), 5-min rest, left cold (LC) 10-min rest, right warm (RW), 5-min rest, and

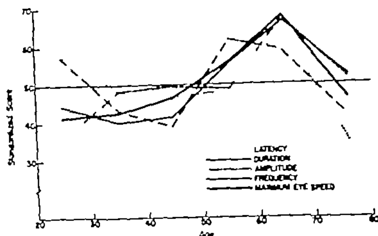


Fig 1 Standard scores ($\bar{X}$ = 50, S D = 10) for nystagmus measures for all four irrigations plotted over age. The latency curve has been inverted for display purposes. The high point for latency for the 60 to 70 group represents decreased or fast latency. The high points for the other measures represent increases.

measure, is adjusted to have a mean of 50 and a standard deviation of 10. As a result, the concordance among the various measures in displaying the age effect becomes *quite clear*. The overall trend is that nystagmus response strength increases to a maximum at 60 to 70 years and declines thereafter. As only four subjects over 80 years of age were tested the final data points do not decline so much as they might have otherwise. Latency and amplitude perturb the shape of the function slightly by manifesting relatively high values for the youngest group. Also amplitude peaks in the 50's instead of the 60's.

The raw score means ($\bar{X}$) and standard deviations (S D) upon which Fig. 1 was based are given in Table _, which provides separate means for the warm and cold irrigations. The male and female nystagmus results were virtually the same and were combined for the means shown in the table. Similarly left-right ear differences were too inconsequential to justify their separate presentation. The data in Table reiterate the tendency for nystagmus responsivity to increase in a roughly regular fashion into the seventh decade (ages 60 to 70). Additionally these data reveal a differential responsivity to the warm and cold stimuli in conjunction with age. The older subjects give disproportionately greater response to the warm irrigations than to the cold.

Significant age-group differences are indicated in Table 2 by superscripts adjacent to the means found to differ by *t*-test comparisons. No significant age differences were observed for latency although latency displayed the same trend as the other measures (Fig. 1). For the cold stimuli, frequency was the only measure to demonstrate significant differences between age groups. For the warm stimuli and the warm and cold combined (total) several significant age differences are shown, particularly for frequency and MES. A few other age-group contrasts were significant at the one tailed level of significance, but these were superfluous to the age differences already evident in Table 2 and are not presented.

The magnitudes of the warm minus cold differences as a function of age are presented in Table 3. The differences in temperature response tend to increase with age in a fairly systematic fashion for duration, amplitude and MES. Between age-group differences on each measure were tested with the *t*-statistic, the outcomes of which are coded in Table 3 by superscripts. In several instances the *t*-test comparisons confirm the significance of the old versus young response differences to the bithermal irrigations.

Fig. displays the age changes for each of the four caloric irrigations using the MES measure. The ordinate scale has been expanded

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Note Only four subjects were over 80 years old, viz., 83 84 86 and 88. All were females.

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Horizontal eye movement potentials were detected via disc electrodes lateral to the outer canthi referred to a forehead indifferent. The signals were led to a Grass P 6 preamplifier (time constant = 1 7 sec) and written out on a Mingograf 24-B oscillograph. Amplitude of the recording pen height was standardized across subjects by incorporating a 10-degree lateral gaze calibration factor determined prior to the first irrigation Paper speed was 10 mm/sec

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Five measures of nystagmus were quantified (1) Latency—time to first beat after start of irrigation (2) Duration—time from first to last nystagmus beat (3) Frequency—number of beats per sec (4) Amplitude—average peak deflection over a series of beats (5) Maximum Eye Speed (MES)—speed of the slow component in degrees per sec = average peak amplitude—average duration of slow component (Coats 1966) For analysis of frequency amplitude and MES, a segment of about 15 beats was selected from the nystagmogram during the period of most intense nystagmus (about 60–90 sec after start of irrigation) Two indices of vestibular symmetry based on MES Unilateral Weakness (UW) and Directional Preponderance (DP) also were calculated (Jongkees, Maas & Philipszoon 1962)

$$\%UW = \frac{(RC+RW)-(LC+LW)}{RC+LC+RW+LW} \times 100$$

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Unilateral weakness represents the percentage difference between left and right labyrinthine responses. Directional preponderance represents the percentage difference between left and right beating nystagmus from both labyrinths.

Hearing tests

Hearing thresholds were determined for each ear using a *Grason-Stadler E 800 Békésy* Audiometer in an IAC 1201 A room. The attenuation rate was 2.5 dB per sec. Pulsed (2.5 interruptions per sec) and continuous tone thresholds were obtained for the frequency range *125–8 000 Hz, and scored by midpoint* interpolation between the peaks and troughs of the tracings.

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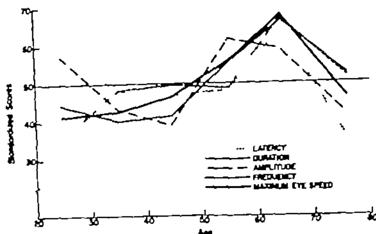


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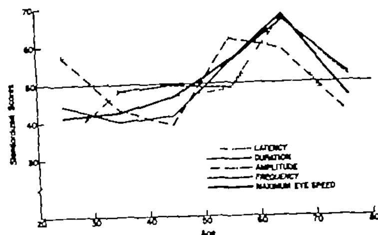


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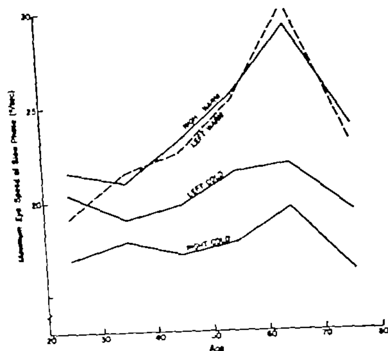


Fig. 2. Raw score means for maximum eye speed of slow nystagmus component plotted over age for each of the four irrigations.

meters (amplitude, frequency and MES_s), Fig. 3 indicates that all age groups tended to give stronger responses to the two warm stimuli than to the cold. Aside from the fact that the older subjects showed a greater warm–cold response difference than the young, which was noted above the present point is that the warm stimuli tended to elicit a greater mean intensity of response than the cold for all our subjects, regardless of age, and this outcome is at odds with some previous reports (Fitzgerald & Hallpike, 1942, and others) To some extent the cold dominance observed by others can be attributed to the corneo-retinal potential shift (Hart, 1969). Regarding the general warm dominance presently observed, however this must in part reflect the unfortunate failure of our apparatus to maintain the cold water bath at less than 31.5 C. The resulting asymmetry being that the cold stimulus was only 5.5 below normal body temperature (37 C), while the warm (44 C) was correctly 7.0 above. A warm dominance in nystagmus intensity would naturally be expected under these circumstances. Preber (1958) and Mehra (1964) however likewise observed a significant MES_s warm dominance (with young adults) using accurately controlled irrigation temperatures (30 and 44 C). While our absolute warm–cold response differences should not be taken at face value, this does not detract from the finding of a progressive increase in the warm–cold response difference up to age 70 The asymmetrical temperatures have presumably only shifted the baseline of the cold response somewhat.

Generalizing from the temperature studies of Henriksson (1956) and Maran (1966) an increment of about 5 /sec in MES_s over the present means for the cold stimuli would have

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Subsequent to the gathering of the present data the cold water temperature control mechanism was corrected to provide the proper degree of coldness (30°C). T elve new subjects, all over 50 years old, ha e since been tested. Eight of the twelve manifested higher MES in response to the tw warm irrigations compared with the cold, indicating that the warm dominance observed was not an artifact of asymmetrical temperature.

Table 2. *Age group mean nystagmus scores for warm and cold stimuli*

Age	20–29 ($n = 36$)		30–39 (59)		40–49 (77)		50–59 (60)		60–69 (42)		70–88 (19)	
	$\bar{X}$	S.D	$\bar{X}$	S.D	$\bar{X}$	S.D	$\bar{X}$	S.D	$\bar{X}$	S.D	$\bar{X}$	S.D
Latency (sec.)												
Cold	24.3	7.7	25.0	9.0	25.2	9.0	25.2	9.8	24.2	8.3	25.6	8.9
Warm	23.3	11.3	25.4	8.8	25.5	10.8	25.3	10.2	23.1	8.6	27.3	11.2
Total	23.8	9.7	25.2	8.9	25.4	9.9	25.2	10.0	23.6	8.4	26.4	10.0
Duration (sec.)												
Cold	124.8	22.6	122.9	25.5	121.2	27.4	127.0	27.5	130.5	28.6	123.8	25.2
Warm	121.5^{a}	27.2	119.6^{b}	28.0	122.4	29.8	128.7	27.8	136.8^{abc}	36.6	130.2	35.5
Total	123.2	24.9	121.2^{a}	26.7	121.8^{b}	28.6	127.8	27.6	133.6^{ab}	32.8	126.8	29.9
Amplitude (deg.)												
Cold	8.4	3.2	7.6	3.0	7.5	3.3	8.3	3.5	7.6	3.2	7.5	2.6
Warm	8.6	3.2	8.1	3.8	7.8^{a}	3.3	9.4	5.7	9.4	4.3	8.1	2.8
Total	8.5	3.2	7.8	3.4	7.6	3.3	8.8	4.9	8.5	3.9	7.8	2.7
Frequency (bts/sec.)												
Cold	1.72^{a}	.42	1.94^{b}	.52	1.89^{c}	.49	1.88^{d}	.49	2.21^{abcd}	.58	1.92	.59
Warm	1.80^{a}	.53	2.01^{b}	.64	2.08	.68	2.07	.59	2.33^{ab}	.76	2.13	.57
Total	1.76	.47	1.98^{b}	.58	1.99^{c}	.60	1.97^{d}	.55	2.27^{abcd}	.68	2.03	.58
Max. Eye Speed (°/sec.)												
Cold	18.6	9.4	18.4	8.1	18.4	9.4	19.5	8.9	20.7	9.1	17.7	7.4
Warm	20.3^{a}	11.5	21.1^{bd}	9.9	22.7^{c}	12.0	25.6^{d}	14.5	29.6^{abc}	15.8	23.1	9.2
Total	19.5^{a}	10.5	19.8^{b}	9.1	20.6^{c}	11.0	22.5	12.4	25.1^{abc}	13.6	20.4	8.8

Note Superscripts designate significant ($p < 0.05$ two-tailed) differences between age groups using t-tests. For a given nystagmus measure (e.g. within a row) age group pairs bearing the same superscript letter are significantly different.

in this figure to exaggerate the differences between *warm* and cold *responses*, which we find are shown more dramatically by MES than by any of the other parameters. The warm hyper reactivity of the 50- and 60-year-olds is prominent in this figure. It is apparent that only *minimal changes with age occur for the cold* stimuli. An additional finding shown in Fig. 2 is the greater MES response by all age groups to the left cold irrigation (which is administered second) relative to the right cold (first) This may be due to an intensification of the corneo-retinal potential following the initial cold calorization as documented by Hart (1969) The amount of difference between the right and left cold calorizations appears to undergo no variation as a function of age in Fig. 2 suggesting that corneo-retinal potential shifts, if they occurred were constant over age.

With respect to the nystagmus intensity para-

Table 3 *Age group mean differences in nystagmus responses to warm and cold stimuli* $LW + RW - LC - RC$

Age	20–29 ($n = 36$)	30–39 (59)	40–49 (77)	50–59 (60)	60–69 (42)	70–88 (19)
Latency	28	66	0	.07	−2.56	1.50
Duration	−3.56	-6.71^{ab}	3.72	4.02	10.31	16.62^{b}
Amplitude	$.45^{a}$	1.05^{b}	$.57^{c}$	1.35^{d}	3.46^{abcd}	1.19
Frequency	18	18	.38	.37	35	42
Max. eye speed	3.34^{ab}	5.46^{cd}	8.64	11.67^{ac}	17.77^{bd}	10.91

Note Superscript letters designate significant ($p < 0.05$, two-tailed) differences between age groups using t-tests. For a given parameter (i.e. within a row) age groups pairs bearing the same superscript had significantly different warm–cold response differences.

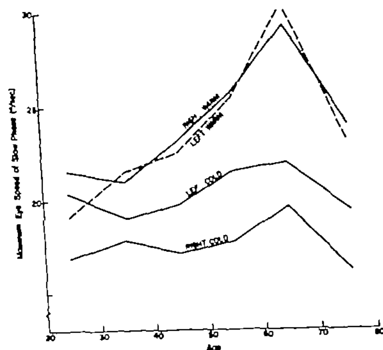


Fig. 2. Raw score means for maximum eye speed of slow nystagmus component plotted over age for each of the four irrigations.

meters (amplitude, frequency and MES.) Fig. 3 indicates that all age groups tended to give stronger responses to the two warm stimuli than to the cold. Aside from the fact that the older subjects showed a greater warm–cold response difference than the young, which was noted above the present point is that the warm stimuli tended to elicit a greater mean intensity of response than the cold for all our subjects, regardless of age, and this outcome is at odds with some previous reports (Fitzgerald & Hallpike, 1942, and others) To some extent the cold dominance observed by others can be attributed to the corneo-retinal potential shift (Hart, 1969). Regarding the general warm dominance presently observed, however this must in part reflect the unfortunate failure of our apparatus to maintain the cold water bath at less than 31.5 C. The resulting asymmetry being that the cold stimulus was only 5.5 below normal body temperature (37 C) while the warm (44 C) was correctly 7.0 above. A warm dominance in nystagmus *intensity* would naturally be expected under these circumstances. Preber (1958) and Mehra (1964) however likewise observed a significant MES, warm dominance (with young adults) using accurately controlled irrigation temperatures (30 and 44 C). While our absolute warm–cold response differences should not be taken at face value, this does not detract from the finding of a progressive increase in the warm–cold response difference up to age 70. The asymmetrical temperatures have presumably only shifted the baseline of the cold response somewhat.[1]

Generalizing from the temperature studies of Henriksson (1956) and Maran (1966) an increment of about 5 /sec in MES over the present means for the cold stimuli would have

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[1] Subsequent to the gathering of the present data the cold water temperature control mechanism was corrected to provide the proper degree of coldness (30°C). Twelve new subjects, all over 50 years old, have since been tested. Eight of the twelve manifested higher MES in response to the two warm irrigations compared with the cold, *indicating that* the warm dominance observed was not an artifact of asymmetrical temperature.

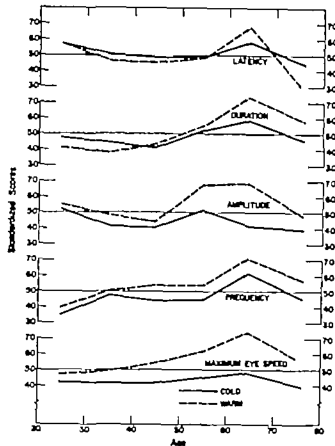


Fig 3 Standard scores for each nystagmus measure plotted over age separately for warm and cold stimuli. The latency curves have been inverted (see Fig. 1 caption).

been observed had the water been 30 rather than 31 5 C Projecting this hypothetical increment onto Fig. 2 produces the inference that our young subjects would have given a stronger MES response to the cold stimuli than to the warm whereas the converse would be true for the 50- and 60-year-olds. Thus, paradoxically the young groups would have shown a response decline" (Stahle, 1956) associated with repeated irrigations, while the middle aged subjects would have shown a response enhancement.

Despite the temperature asymmetry Fig. 3 shows that for latency and duration the young subjects still demonstrated higher scores in response to the cold water compared with the warm. The ability of the cold dominance to override the temperature asymmetry suggests that this response bias is a potent one at least for latency and duration. It is not surprising then that the duration measure has been frequently noted to reflect higher values for cold as opposed to warm irrigations when the cold water was correctly 30 C (Fitzgerald & Hallpike, 1942) In accord with the present results Preber s (1958) and Mehra s (1964) young adults showed significantly longer durations following cold stimulation while showing significantly greater MES following warm. As the cold dominance was seen only in our younger subjects and since relatively young subjects have been typically used in previous studies, it may be that the traditional prolongation of nystagmus following cold stimuli relative to warm does not occur for older subjects. Our results suggest that it does not.

The possibility that the order of administration of the bithermal tests interacts with aging effects must also be considered in the present context. As the warm irrigations are always performed last, it is possible that the aged give a heightened response to them because of some factor associated with repeated testing, to which they are more sensitive than the young. Stahle (1956) varied the caloric test sequence giving one group of subjects the warm calorics first and another group the cold calorics first. He found no important effects of sequence, but age as a variable was not studied. Since the now widely standardized caloric test battery routinely provides the cold irrigations first, the question of age and sequence interaction seems academic.

With regard to response changes following repeated calorics in relation to age Gramowski & Unger (1969) noticed a greater decline (habituation) in 60- to 70-year-old subjects than in 15 to 25-year-olds receiving five cold irrigations at eight minute intervals. All subjects in that study showed some response decline upon repeated testing. From this source we might have anticipated a reduced warm response by our older subjects because the warm calorics were always administered last As the outcome was quite the reverse (i.e enhanced warm response) test repetition can probably be ruled out here as unimportant.

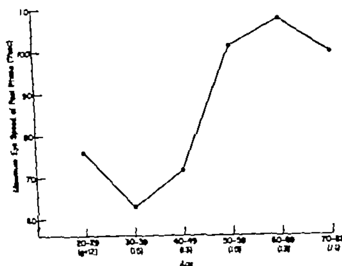


Fig. 4. Raw score mean maximum eye speed of the fast component for the left warm stimulus plotted over age for a subsample of 79 subjects.

FAST PHASE CHANGES

We also examined the speed of the fast phase of nystagmus (MES_f) for age effects. This measure has been previously noted to be insensitive to such manipulations as altering caloric stimulus temperature (Torok & Derbyshire, 1968). MES_f was derived in similar fashion to MES_s, the only difference being that duration of the fast phase was substituted for slow phase duration in the exact method formula (Coats, 1966). As the MES_f measure was not determined routinely for all patients only a subsample was drawn for this purpose.

Fig. 4 presents, for the left warm stimulus, the MES_f age-group means for 79 patients selected at random from our total sample. It is obvious in Fig. 4 that after age 50 the MES_f increases sharply. The significance of the over under 50 difference was confirmed by a Median test (Dixon & Massey 1957). For the cold stimuli, as previously observed for MES_s, age related changes in MES_f were minimal. MES_f and MES_s are not identical reflections of one another however since their intercorrelation was only $r = 0.59$ ($N = 79$). The intensification of nystagmus during middle age can thus be discerned from all measurable aspects of the nystagmogram following warm stimulation.

Table 4. *Asymmetry and spontaneous nystagmus as a function of age*

	Age	20–29 (n = 36)	30–39 (59)	40–49 (77)	50–59 (60)	60–69 (42)	70–88 (19)
Unilateral Weakness	$\bar{X}$	14	2.20	−2.24	−3.54	−2.96	−3.12
	S.D.	15.26	13.00	13.80	11.84	11.22	15.24
Directional Preponderance	$\bar{X}$	7.58	.32	3.18	4.52	−1.94	4.56
	S.D.	14.72	14.96	17.22	15.94	16.74	16.04
Percentage showing spontaneous nystagmus		28	36	42	40	36	42
Percentage showing positional nystagmus		31	29	25	37	33	26

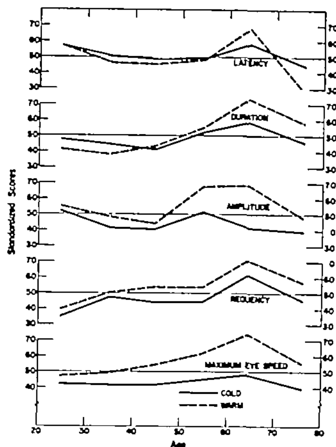


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($r \approx 0.50$). High frequency loss increases in orderly fashion with age.

For caloric nystagmus, however the vestibular age changes are not so orderly The youngest (20's) and oldest (70+'s) groups were shown to form "hooks on the ends of some of the curves in Figs. 1 and 3 Such deviations from linearity tend to reduce the size of linear correlation coefficients (Pearson r) computed between age and nystagmus. These coefficients, which are given in Table 5 for each irrigation, were therefore determined omitting the 70+ group. The exclusion had the effect of raising the coefficients slightly as would be expected. In Table 5 the most consistent age nystagmus correlations occur for frequency Duration and MES, also show some significant r s. The significant age MES correlations under the warm conditions appear associated with the small positive r s demonstrated by amplitude. (MES is computationally dependent on amplitude.) The rises in MES, and amplitude under the warm conditions were also illustrated by the curves for the 50- and 60-year-olds in Fig. 3

Table 5 *Intercorrelations between age and nystagmus measures for each irrigation*

	RC	LC	RW	LW
Latency	−.01	.01	−.03	−.04
Duration	.16	.11	.19	.17
Amplitude	−.02	−.02	.09	10
Frequency	.24	.21	.23	17
MES_v	.08	.07	19	.27

Note: Age 70+ group omitted. Significant ($p < 0.05$) r coefficients are indicated by asterisks.

Intercorrelations were then computed between the nystagmus measures and hearing thresholds, with the 70+ group omitted. Since both nystagmus frequency and hearing loss were known to correlate with age, the age effect was held constant via the partial correlation technique (Hays, 1963) As a consequence, the hearing-nystagmus intercorrelations vanished in all but one instance For the male subjects only at tone frequencies at and above 2 kHz, small but significant partial correlation coefficients ($r \approx .20$) remained between hearing threshold and nystagmus frequency as well as MES_v, with age held constant. The positive sign of these coefficients indicated that the greater the high frequency hearing loss, the greater the frequency and MES of caloric nystagmus (for males up to 70 years old) for both warm and cold stimuli.

This finding was examined more closely by comparing subjects showing the most bilateral high frequency hearing loss (2 kHz − 8 kHz) with those showing the least, with respect to MES_v. This was performed separately for males and females of all ages up to 70 years old. The most- and least-loss subgroups were matched for age, with their n s determined by the number of subjects available with sufficient hearing loss. Table 6 gives the mean MES_v of all four irrigations and mean hearing threshold levels at 2 kHz − 8 kHz for the selected male subjects. It is evident in Table 6 that males with high frequency loss tend to show greater MES than no-loss males. This difference was significant for all ages combined ($t = 2.28$ $p < .05$). MES shows roughly a 5 / sec increase for the poor hearing subjects over 50 years old relative to those under 50 Very little such increase is apparent for the normal-hearing men. High frequency hearing loss appears associated with intensified nystagmus for men of all ages up to 70. The corresponding analysis done for females revealed no significant most–least hearing differences in MES_v (These data are not tabulated here.) However the females degree of hearing loss was clearly much less than that of the males. The pattern of severe high frequency hearing loss and intensified nystagmus among males may *represent* a characteristic sex difference.

The associated change in hearing and nystagmus shown by men with high frequency hearing loss implies a deteriorative effect common to both cochlear and vestibular end organs not dependent upon age. If we assume that the high frequency hearing losses observed were solely the result of acoustic trauma (noise) this leads to the speculation that acoustic trauma,

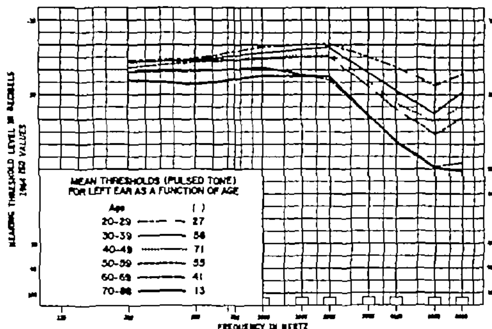


Fig 5 Mean Békésy pulsed-tone thresholds (left ear) *for each of the six age* groups. Some *N*s are reduced due to missing audiograms.

SYMMETRY AND SPONTANEOUS NYSTAGMUS

Table 4 gives the mean values for unilateral weakness (*UW*) and *directional preponderance* (DP) as well as as the incidence of spontaneous and positional nystagmus for each age group There are no systematic differences in UW or DP as a function of age The formulae for UW and DP (see under Method) are such that the negative UW values shown in Table 4 indicate a right side weakness for all age groups while positive DP values *(preponderance to the* right) occur for all but the 60-year-olds. This *consistent right asymmetry may* be attributed to the heightened nystagmus response provoked by the second cold irrigation (LC) relative to the first (RC) as was illustrated in Fig. 2 Applying the formula for DP the enhanced response to the left cold irrigation has the effect of inflating the right beating nystagmus score, yielding a right DP At the same time for UW the left side total score (LC + LW) becomes inflated yielding a right weakness. As mentioned earlier this rise has been explained by Hart (1969) as *due to a shift in* the corneo-retinal potential following the first irrigation. Since the responses to the two warm irrigations did not differ (Fig. 2) they had no effect on the *asymmetry measures*.

Table 4 also gives the percentage of subjects in each age group manifesting spontaneous or positional nystagmus as defined by Coats (1966) There are no differences among the various age groups in these percentages. The incidence of spontaneous ($\bar{X} = 37\%$) and positional ($\bar{X} = 30\%$) nystagmus observed in this sample is in reasonable agreement with the incidence (36% and 35% respectively) reported by Coats (1966) for similar subjects. In 64% of our subjects manifesting spontaneous nystagmus the quick phase direction was to the right. This would account in part for the right DP tendency observed (cf Coats, 1966)

INTERCORRELATIONS HEARING CALORIC NYSTAGMUS AND AGE

The Békésy hearing thresholds of our patients are plotted as a function of age in Fig. 5 revealing the traditional decline *in high fre*quency hearing acuity with age (presbycusis) Correlation coefficients computed between hearing thresholds and age indicated the highest coefficients to be at 6 kHz and 8 kHz

Table 7 *Intercorrelations among measures of nystagmus for cold and warm irrigations*

		Duration	Amplitude	MES_v	Frequency
Latency	C	.443	−.217	−.162	−.008
	W	−.396	−.235	−.253	−.011
Duration	C		.276	.272	.060
	W		.225	.190	.125
Amplitude	C			.628	−.292
	W			.734	−.131
MES_v	C				.270
	W				.514

Note: Nonsignificant *r*'s are indicated by asterisks. All others were significant ($p < 0.05$).

coefficients obtained between frequency and amplitude. Accordingly as frequency increases amplitude tends to decrease, and vice versa. Frequency and to a minor extent, amplitude were previously noted to increase slightly with age up to the 70's, at least for the warm stimuli. But the possibility of both measures increasing is obviously limited due to the reciprocal condition mentioned.

Table 7 indicates some degree of independence from the other measures for the frequency parameter. Latency and duration are not at all correlated with frequency although they are mildly correlated with amplitude and MES_v. And while frequency is intercorrelated with amplitude and MES_v, the magnitude of the coefficients varies from cold to warm. The latter fact could reflect changes in the central control of the interaction between slow and fast phases as a function of stimulus temperature. The cold and warm irrigations may engage slightly different reflex arrangements mediating the rhythmic balance between slow and fast components. Torok (1969) found that the correlation between frequency and MES_v decreased as caloric temperature was reduced from 30 to 10 C. It would be of interest to determine if a similar divergence occurs for varying degrees of hotness.

IV Literature Review and Critique

In retrospect, it appears clearly possible to assemble most of the earlier studies, which at first appeared quite diverse in their outcomes, and reconcile their findings into accord with the picture developed by the present data. The key to reconciling the various findings lies chiefly with how the subjects were grouped by age and what age range was investigated. Shortcomings in data analysis appeared a frequent problem while variations among techniques of vestibular testing seemed relatively unimportant.

The present and the previous studies have employed exclusively the cross-sectional approach to studying age effects on vestibular function. In this approach, groups of subjects born at different times are compared. To date, no studies in this area have used a longitudinal design, wherein individuals are compared with themselves as they age. An unavoidable problem associated with cross-sectional studies is that differences may exist between populations of subjects born at different times which are attributable to factors other than the aging process. Thus in the present study where we compare subjects born in 1950 with those born in 1900, we must recognize that many differences may exist between the two age groups with respect to their different life experiences (e.g., diet,

Table 6. *Mean MES and hearing levels (2k–8kHz) of males selected for least high frequency hearing loss*

	Age groups				
Hearing loss	20–39 (n=24)	40–49 (20)	50–59 (14)	60–69 (12)	All (70)
MES$_s$					
Most	20.40	19 61	25.97	26.80	22.39
Least	15 47	19.33	19.37	18.32	17 84
Hearing levels (dB)					
Most	38.9	47.5	45 8	64.5	47 1
Least	8.2	10.2	20.0	35 4	15 8

Note Age group *N*'s should be divided by two to find Most or Least subgroup *n* s. The 20 s and 30's were combined due to a small number of 20's having hearing loss.

which is relatively uncommon in women can bring about degenerative changes in the vestibular apparatus as well as the cochlea. In an earlier study on lateralization to be described below we similarly noticed vestibular hearing relations among men with high frequency hearing losses (Bruner & Norris, 1970) However the assumption that high frequency hearing loss is produced solely by unusual noise exposure may not always be warranted since similar audiograms are often associated with acute inner ear infections, presumably viral (D E Kilgore M D Lovelace Clinic, personal communication) In such a case, ordinary noise levels may be able to induce transient or permanent high frequency loss in the vulnerable cochlea.

Except for the association between high frequency loss and nystagmus intensity in men no further relations could be established between hearing and nystagmus in the total sample This included an analysis of threshold differences between continuous and pulsed tones. In Ménière s patients, Enander & Stahle (1969) have reported that a large continuous pulsed separation is associated with caloric hyporeactivity In the present study the separation between continuous and pulsed tracings tended to increase with age at the high frequencies But the separation was not correlated with nystagmus intensity when the age influence was held constant.

In a previous study (Bruner & Norris, 1971) involving test pilots and patients presenting Békésy audiograms indicative of unilateral acoustic trauma (i.e. a high frequency notch) we obtained a significant correlation between the extent of bilateral hearing threshold differences and caloric DP where the direction of preponderance was toward the worse hearing ear at high frequencies. The lateralization of DP and hearing loss was not evident in patients having wideband unilateral hearing loss or in normals with bilaterally symmetrical hearing acuity In the current heterogeneous sample of patients, no correlation was found between DP and unilateral high frequency loss. Thus, the impression is continued from the earlier study that a unilateral acoustic trauma type audiogram may be essential for the lateralization effect to appear But as discussed above, a history of severe noise exposure may not be essential for this phenomenon.

INTERCORRELATIONS BETWEEN NYSTAGMUS MEASURES

The intercorrelations between the several nystagmus measures are presented in Table 7 for both cold and warm stimuli. Latency and duration are moderately intercorrelated indicating that subjects having brief latencies tended to show prolonged durations Subjects having brief latencies and long durations also tended to have high amplitudes and high MES although the degree of association is minor

Frequency and amplitude are intercorrelated positively with MES$_s$ that is, as either of the former increases so does MES$_s$. This is a necessary outcome from the manner of deriving MES$_s$ which incorporates both amplitude and beat duration (= reciprocal frequency) These three parameters are viewed as indices of nystagmus intensity or vigor But the nystagmus jerk which terminates each complete beat cycle must, at the same time limit the extent of slow phase travel (amplitude) This reciprocal relation is illustrated in the intercorrelation matrix (Table 7) by the negative signs of the small

jects in each age group was not specified, only that a total of 89 subjects was divided into five groups (ages 0–10 10–20, 20–30 30–40 and 40–70). The oldest group (40 to 70) presumably included only about 18 subjects. From the present study we would expect that both high and low points of nystagmus strength could occur within the 40 to 70 group of Minnigerode et al., however their grouping procedure would obfuscate the possibility of such a finding. Furthermore, inspection of the data curves reveals that the oldest group demonstrated slightly greater mean scores for nystagmus duration, amplitude, and number of beats than the 30- to 40-year-olds. Thus the authors claim of a systematic response diminution with age seems questionable, since some indications of a middle-old-age peak were found.

Haas (1964) claimed to have found increased mean rotatory thresholds for normal subjects between 51 and 80 years of age relative to 15- to 50-year-olds. When grouped in this manner the data support the claim, but again grouping obscures precision. Where Haas data are presented in decade form they indicate that the 41–50 group yielded the most sensitive thresholds of all groups, whereas the 51–60 group appears to have even higher thresholds than subjects over 60 To complicate matters further some of Haas age decades contained fewer than 10 subjects, which severely jeopardizes the reliability of his group means.

Camarada & Lumia (1959) used rotational stimuli to assess vestibular sensitivity in 23 subjects aged 70 to 92 years. They found higher thresholds than those considered normal for young subjects. This loss of vestibular excitability in very old people is consistent with most other findings. But it is important to note that age effects as shown by tests of vestibular excitability (rotational or caloric thresholds) may not necessarily follow the same time course of change as when testing responsivity (nystagmus duration, intensity etc.). Thus it is not inconceivable that middle-aged subjects may require relatively strong stimuli to elicit nystagmus initially yet respond with hyperactive nystagmus once the threshold has been surpassed.

Okano (1938) tested 223 healthy subjects ranging in age from the 60's through the 80's using both rotational and caloric stimuli. Nystagmus duration and number of beats decreased gradually with age, and latency tended to increase. Okano s results are in accord with most others in finding a vestibular response decrement after the seventh decade.

Brookler & Pulec (1970) observed a decrease in MES, with age using cold irrigations, but no change using warm. Their curve for the right warm stimulus indicates, however that beginning with the 20-year-olds, MES increased gradually to a peak at the 65- to 74-year age group. The curve then declines for the 80's before rising again for the 90's. (The 90-year data point is probably unreliable since it is based on only six subjects.) The gradual increase in MES up to the 70's for the right warm stimulus displays the same function as seen in the current study Furthermore, the curve drawn by Brookler & Pulec would have been steeper and possibly significant, had they employed the exact method for determining MES (Coats, 1966) instead of their approximate method (measuring fast phase amplitude) which underestimates the exact method by about 20% (Stahle, 1956 Henriksson, 1956). Thus at least for one of their warm irrigations, Brookler & Pulec witnessed results similar to our own.

Brookler & Pulec's (1970) decreasing responsivity with age for the cold stimuli may be a result of including patients with known pathology into their sample. Apparently over 80% of Brookler & Pulec s sample fell into one or more diagnostic categories, including Ménière s disease (16 4%). Although an age distribution for each diagnostic category was not provided, it is probably safe to assume that the incidence of pathology increased with age. As labyrinthine pathology is usually evidenced by caloric hypoactivity from one or both ears (e.g., Fig. 2 of Brookler & Pulec, 1970), the inclusion of a substantial proportion of pathologies would

The principal aging effects reported in the literature appeared to fall conveniently into three classifications (1) Hypo-reactivity (2) Hyper-reactivity or (3) no change with age. The studies will be reviewed in terms of these three categories for ease of presentation, recognizing that some of the studies may actually have observed (or overlooked) an increase followed by a decrease in nystagmus responsivity with increasing age.

Hypo-reactivity with age

Arslan (1957) evaluated vestibular reactions in a group of 50 non-vertigo patients ranging in age from 49 through 84 years using Viet s method of cold water stimulation. For nystagmus duration and number of beats 30 of the 50 patients scores fell below one standard deviation of a norm group of 100 healthy 20-year-olds tested earlier (Arslan, 1955) The author concluded that a shift toward vestibular hypo-excitability"[1] occurs in the elderly

Further study of Arslan s data (1955 1957) suggests that his conclusion requires some qualification. Thirty-one of the 50 subjects in Arslan s old group were over 70 years of age These 31 people manifested low nystagmus scores which had the effect of depressing the means for the entire group below those of the 20-year-olds. When Arslan s 50- and 60-year olds are considered separately their means for number of beats ($\bar{X} = 109.0$ and 106.5 respectively) are larger than the mean of the 20-year olds ($\bar{X} = 94.4$) Thus in agreement with the current findings, Arslan s middle-aged subjects appear hyper reactive relative to the 20-year olds. His conclusion of "hypo-excitability for number of beats applies correctly only for his subjects over 70 years of age For the duration measure Arslan s young group did manifest higher values than his old group But aside from the age-grouping problem here, it has been found previously that, in contrast to older subjects the young may respond with longer durations to cold stimulation yet respond with weaker intensities to warm (Preber 1958 Mehra, 1964)

Zelenka & Slaninova (1964) concluded that the senile vestibular apparatus has a diminished reactivity to rotational as well as caloric stimulation. Tested were 396 healthy subjects ranging in age from 5 to 79 years. From Zelenka & Slaninova s tabulated data it is apparent that after about 20 years of age the nystagmus responses (latency duration threshold and MES,) began to intensify with age attaining maximum reactivity in the 40 s and 50 s for both types of test stimulation. After age 60 responsivity tended to decline, which presumably was the basis for their conclusion of senile hypo-reactivity

Rossberg (1964) using rotational stimuli in three groups of healthy subjects (20–40 41–60 and 61–80 years of age) observed decreases with age in nystagmus amplitude, number of beats and to a lesser extent, duration. This outcome is contradictory to the present findings. Rossberg s three age groups each spanned 20 years and each contained only 20 subjects. His broad grouping and small *n* s preclude evaluation of fine age differences. For example, one cannot determine how many of the 61 to 80-year-old subjects were over 70 We can only suggest that the reliability of Rossberg s observations be verified by additional studies using his technique (cupulometry)

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quality of medical care, attitudes toward exercise etc.) associated with cultural change plus possible genetic changes, which will inevitably confound the pure effects of age. Analogous difficulties accompany purely longitudinal designs. Both approaches require sophisticated design and analysis to separate out the age factor (Schaie, 1965 Wohlwill 1970)

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jects in each age group was not specified, only that a total of 89 subjects was divided into five groups (ages 0–10 10–20 20–30, 30–40 and 40–70). The oldest group (40 to 70) presumably included only about 18 subjects. From the present study we would expect that both high and low points of nystagmus strength could occur within the 40 to 70 group of Minnigerode et al., however their grouping procedure would obfuscate the possibility of such a finding. Furthermore, inspection of the data curves reveals that the oldest group demonstrated slightly greater mean scores for nystagmus duration, amplitude, and number of beats than the 30- to 40-year-olds. Thus the authors claim of a systematic response diminution with age seems questionable, since some indications of a middle-old-age peak were found.

Haas (1964) claimed to have found increased mean rotatory thresholds for normal subjects between 51 and 80 years of age relative to 15- to 50-year-olds. When grouped in this manner the data support the claim, but again grouping obscures precision. Where Haas data are presented in decade form they indicate that the 41–50 group yielded the most sensitive thresholds of all groups, whereas the 51–60 group appears to have even higher thresholds than subjects over 60. To complicate matters further some of Haas age decades contained fewer than 10 subjects, which severely jeopardizes the reliability of his group means.

Camarada & Lumia (1959) used rotational stimuli to assess vestibular sensitivity in 23 subjects aged 70 to 92 years. They found higher thresholds than those considered normal for young subjects. This loss of vestibular excitability in very old people is consistent with most other findings. But it is important to note that age effects as shown by tests of vestibular excitability (rotational or caloric thresholds) may not necessarily follow the same time course of change as when testing responsivity (nystagmus duration, intensity etc.). Thus it is not inconceivable that middle-aged subjects may require relatively strong stimuli to elicit nystagmus initially yet respond with hyperactive nystagmus once the threshold has been surpassed.

Okano (1938) tested 223 healthy subjects ranging in age from the 60's through the 80's using both rotational and caloric stimuli. Nystagmus duration and number of beats decreased gradually with age, and latency tended to increase. Okano's results are in accord with most others in finding a vestibular response decrement after the seventh decade.

Brookler & Pulec (1970) observed a decrease in MES with age using cold irrigations, but no change using warm. Their curve for the right warm stimulus indicates, however that beginning with the 20-year-olds, MES, increased gradually to a peak at the 65- to 74-year age group. The curve then declines for the 80's before rising again for the 90's. (The 90-year data point is probably unreliable since it is based on only six subjects.) The gradual increase in MES up to the 70's for the right warm stimulus displays the same function as seen in the current study. Furthermore, the curve drawn by Brookler & Pulec would have been steeper and possibly significant, had they employed the exact method for determining MES (Coats, 1966), instead of their approximate method (measuring fast phase amplitude) which underestimates the exact method by about 20% (Stahle, 1956 Henriksson, 1956). Thus at least for one of their warm irrigations, Brookler & Pulec witnessed results similar to our own.

Brookler & Pulec's (1970) decreasing responsivity with age for the cold stimuli may be a result of including patients with known pathology into their sample. Apparently over 80% of Brookler & Pulec's sample fell into one or more diagnostic categories, including Ménière's disease (16.4%). Although an age distribution for each diagnostic category was not provided, it is probably safe to assume that the incidence of pathology increased with age. As labyrinthine pathology is usually evidenced by caloric hypoactivity from one or both ears (e.g. Fig. 2 of Brookler & Pulec, 1970) the inclusion of a substantial proportion of pathologies would

The principal aging effects reported in the literature appeared to fall conveniently into three classifications (1) Hypo-reactivity (2) Hyper-reactivity or (3) no change with age. The studies will be reviewed in terms of these three categories for ease of presentation recognizing that some of the studies may actually have observed (or overlooked) an increase followed by a decrease in nystagmus responsivity with increasing age.

Hypo-reactivity with age

Arslan (1957) evaluated vestibular reactions in a group of 50 non vertigo patients ranging in age from 49 through 84 years using Veit s method of cold water stimulation. For nystagmus duration and number of beats, 30 of the 50 patients scores fell below one standard deviation of a norm group of 100 healthy 20-year-olds tested earlier (Arslan, 1955) The author concluded that a shift toward vestibular hypo-excitability"[1] occurs in the elderly

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tend to reduce the mean level of nystagmus response strength in conjunction with age. Unfortunately Brookler & Pulec did not present a separate analysis of age effects for their 839 patients considered free of neuro-otologic disease. While Brookler & Pulec s aging findings (i.e. hyporeactivity) may be representative of the total, unscreened patient population seen in an ENT clinic, we suggest that our own findings (i.e. peak hyper reactivity) are more typical for patients relatively free of pathology

Hyper-reactivity with age

Kotyza (1939) studied vestibular function in 117 healthy subjects between the ages of 11 and 63 years, using both rotational and caloric stimuli. With the rotational stimulus, nystagmus duration and number of beats increased up to 45 years of age and then declined slightly in older subjects Using Kobrak s minimal cold caloric test, the number of beats was observed to increase up to age 50 and decline thereafter while latency and duration showed little change The author concluded that the labyrinth is most responsive in the fifth decade. In addition to his finding of peak responsivity in the middle decades, Kotyza s caloric results are similar to our own in that frequency was the parameter most sensitive to age influences using cold stimuli

Guedry (1950) determined the duration of subjective movement sensations following rotation in two groups of 24 subjects each One group ranged in age from 19 to 21 years, and the other from 30 to 53 years. The older group s response indicated a significantly longer duration of perceived movement. This result indicates heightened vestibular effects for Guedry s older subjects which agrees with the present view As one of several possible explanations for his finding, Guedry conjectured that older subjects may have less central control over the vestibular reflex system" (p 3) This notion will be discussed at length later in the present

Chladek (rotational and cold caloric test. tion of 51 hearing-loss patients aged 60 to 75 The author s summary claimed that suprathreshold post rotational excitability was frequently raised the caloric reaction was, however reduced." Chladek compared his results with control data apparently borrowed from Zelenka although no specific reference to a published source of Zelenka s data was cited (Both men were affiliated with the same institution.) No statistical tests were mentioned. When compared with Zelenka s control data Chladek s subjects reveal prolonged durations of post rotational nystagmus, in support of the first claim in his summary With respect to the caloric results, however the proximity of the means of the experimental and control groups and the large size of the standard deviations suggest no significant differences.

In comparison with some normative data published earlier by Zelenka & Slaninova (1964) Chladek's (1966) subjects do indeed reveal prolonged post rotational durations. The caloric results do not, however appear re duced" But this may simply point up the inherent difficulties in trying to compare accurately the data of different investigators. In sum there is some indication that Chladek s subjects revealed post rotational hyper reactivity but none that their caloric responses were hyporeactive. As Chladek sampled only old subjects, about half of whom had severe hearing losses, his findings hold limited value for the present purposes.

Gramowski & Unger (1969) compared a group of 10 normal males between 15 and 25 years old with eight men between 60 and 70 for age differences in habituation to cold calorics Five irrigations of 30 C water were administered at eight minute intervals to the right ear On all measures of nystagmus the old group showed greater habituatory decrement (i.e response decline from first to last irrigation) than the young group *Additionally the* older men manifested longer nystagmus durations. The authors interpreted these observations to mean that active, youthful subjects exhibit some level of habituation even prior to

experimental stimulation. As a consequence of aging, this pre habituation supposedly dwindles, resulting in heightened initial response levels and greater response declines. A lessening of central inhibition would seem the likely neural correlate of this process.

Most important for the present findings is the fact that Gramowski & Unger's 60- to 70-year-olds yielded longer nystagmus durations than the young on every irrigation, most of all on the first one. Unfortunately except for the duration scores, the age-group differences for the other nystagmus measures were not given.

No change with age

Bourlière (1948) tested 160 women ranging in age from 20 to 84 years, using galvanic stimulation to elicit head movements. He concluded that vestibular excitability is not affected by age. However Weiss (1959) performed *t*-tests on Bourlière's published data and found significant between-group differences indicative of vestibular hypo-excitability after the sixth decade. Further inspection of Bourlière's tabulated data reveals that vestibular excitability was slightly higher in the fifth and sixth decades than the fourth, suggesting a two-phased aging function similar to that seen in the present study wherein vestibular responsivity peaks in middle age.

Forgacs (1957) tested 80 nursing home residents over 55 years of age using both rotational and caloric threshold tests. He reported "normal" vestibular reactions in all cases. Forgacs admitted, however that since no normative data were available for comparison with his aged group his conclusion of normality would have to remain tentative until additional subjects could be tested. No subsequent work on this topic by Forgacs was found.

Fregly & Graybiel (1970) administered threshold caloric tests to 76 vertiginous patients ranging in age from 20 to 72 years. The authors mentioned that caloric threshold levels were independent of age although no specific analysis was described. As Fregly & Graybiel's study was not specifically designed to investigate aging, the sample size and age distribution were rather inadequate to determine age effects.

Nystagmus tests in children

Zelenka & Slaninova (1964) examined children using caloric and rotatory techniques in an extensive aging study the adult results of which have already been described. Their sample included 12 children between 5 and 10 years old, 55 between 11 and 15 and 32 young people from 16 to 20 years. According to the authors, the two youngest groups yielded results clearly indicative of "hyporeflexia" relative to adults, particularly for children over seven years of age. Response stabilization and the transition to a normal adult record took place at about age 18. As mentioned earlier the adult age changes observed by Zelenka & Slaninova (1964) revealed an increase in nystagmus to peak reactivity in the middle decades followed by a decline after age 60 Very similar caloric and rotatory findings were obtained for adults by Kotyza (1939), who likewise reported relatively hypo-reactive scores for teenagers.

Young children were also tested by Tibbling (1969) who found that amplitude and eye speed of rotatory nystagmus were most intense in very young children and tended to decrease with age up to 15 years, which was the oldest age studied. The older children in Tibbling's sample presumably correspond to the children over seven years old in Zelenka & Slaninova's sample who were considered hyporeflexive. From these studies and the present one, it appears that the shape of the complete age function for vestibular reactivity is complex. Beginning with infants and young children, the curve reveals hyper-reactivity then decreases to a low point in the second decade, followed by an increase to peak responsivity in the middle decades, and finally depicting hypo-reactivity again in old age—another instance where senility recapitulates childhood.

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Wright, 1967). When subjects are allowed to become drowsy or inattentive, nystagmus may vanish for periods of time following caloric stimulation. Mental alertness tasks tend to eliminate these periods of "suppression" From the concept of inhibitory release, one might predict that suppression is less likely to occur in middle aged subjects than in the young. This could easily be tested by employing mental tasks as the independent variable with old and young subjects.

In addition to inhibitory changes we also have much evidence that aging results in the depression of neural activities characterized mainly by excitation. Sensory thresholds (e.g., hearing levels) are perhaps the prime example. Therefore, when neural systems are classified simply as either excitatory or inhibitory an age-related failure in function may be manifested by superficially opposite effects excitatory activities will be depressed while inhibitory activities will be released.

The notion of a loss of central inhibitory control as the basis for heightened vestibular responsivity associated with either lesions or aging accommodates well to the data. However it can be verified only by corroborating neurophysiological and anatomical evidence, and this is lacking. In the present case we cannot say whether we have observed a central or an end organ effect, or both. The observed changes could be equally well attributed to the labyrinth or to the brain stem (or higher) as both slow and fast components of nystagmus were *affected*.

An obvious etiology for age related changes in vestibular function, whether central or peripheral, would be *vascular insufficiency* an ever present developmental disorder to which the labyrinth is especially prone. Along these lines an early precedent exists for the present findings in the work of Pallestrini (1933) and Scoden (1947), who proposed an explanation based on arteriosclerotic changes To quote from Arslan (1957):

> *Pallestrini* divided arteriosclerosis of the labyrinth into stages: the stage of angioneurosis and that of angiosclerosis. Thus *Scoderi* and *Pallestrini* found in the majority of cases of old subjects belonging to the angioneurotic stage, an increase of vestibular reflexes, above all on thermal stimulation, which is manifested by shortening of the latency period and an increase in the strength of the nystagmic jerk. In the angiosclerotic stage, however a decrease of vestibular reflexes is always found, which is revealed with the lengthening of the latency period and the shorter duration and amplitude of the nystagmic jerk (p. 477).

Zelenka & Kozak (1963) examined 152 patients presenting varying stages of hypertensive and sclerotic disease, ranging from vasospasm ("angioneurosis") to frank arteriosclerosis. All tended to show post-rotatory hyper-reactivity The vestibular neurons which suffer earliest from the ischemia of age and disease appear to be those mediating inhibitory activities.

Local vascular influences

Jongkees (1948) succeeded in reducing nystagmus duration following cold stimulation by local administration of a vasodilator (amyl nitrite) to counteract vasoconstriction. Appropriately the drug had no effect on the hot response since dilation was presumably already maximal. Jongkees further confirmed the importance of local vascular influences on the caloric response with demonstrations of the vasoconstrictive effects of procaine hydrochloride.

The possible involvement of local vascular influences could be a further basis underlying the differences obtained between our middle aged and young subjects. Assume that with age there is a gradual diminution in blood flow to the labyrinth due to vascular narrowing. Applying hot water brings about vasodilation and an improved blood supply which elevates the neural discharge of the stimulated ampulla over that resulting solely *from* cupular bending. It is proposed that the vasodilatory action is more marked in the middle aged ear where the vessels are partially narrowed, than in the young ear where they are fully patent. We are therefore suggesting that the hot caloric *is* in effect a stronger stimulus for middle-aged subjects than for young ones. In the instance of

V Comment and Conjecture

Fourteen studies designed chiefly to investigate the effects of aging on vestibular reactivity have been reviewed and evaluated with reference to the present observations. Nine of these reports we interpret to be in accord or at least not inconsistent, with the current conception of peak responsivity in middle age followed by hypo-reactivity in senility viz. Arslan (1957) Bourlière (1948) Camarada & Lumia (1959) Chladek (1966) Gramowski & Unger (1969) Guedry (1950) Kotyza (1939) Okano (1938) and Zelenka & Slaninova (1964) Of the five remaining studies which did not afford ready concordance those of Haas (1964) Minnigerode Grohmann & Vontin (1967) and Rossberg (1964) were marred by age-group imprecision that of Forgacs (1957) lacked a norm group and in the case of Brookler & Pulec (1970) hypo-reactive pathologies were sampled. The latter five studies therefore appear only weakly contradictory

The age function for adult vestibular behavior which we have described does not portray a simple parallel to hearing. High frequency hearing loss proceeds rather directly with age yielding a strong linear correlation. Such is easily interpreted in terms of gradual tissue aging and cell death (presbycusis) and "wear and tear" (sociocusis, Glorig & Nixon 1962) Vestibular age decrement in the adult describes a response process which at first appears irritative before becoming depressed. Two highly speculative explanations for this process will be offered below (1) Inhibitory Release, and (2) Local Vascular Influences

Inhibitory release

Several CNS "centers" have been clearly implicated in the inhibitory modulation of vestibular responses (Gernandt, 1967) McCabe (1965) has proposed that the limits of the slow phase sweep of nystagmus are regulated by inhibitory neurons in the reticular formation. A relaxation of the latter would permit increased amplitudes and MES. Chladek (1966) noticed hyper reactivity upon testing subjects over 60 years old and commented "The finding of an increased reaction to vestibular stimulation in old age is often without doubt, in connection with the decreased inhibitory activity of the central vestibular neurons" (p 15)

A hyper reactive caloric response is often said to indicate a pathological failure (release) of an inhibitory mechanism within the vestibular system particularly its central portions (Torok & Derbyshire, 1968) In this connection the observed influence of age on caloric nystagmus mimics the effect traceable to a central lesion This is a frequent state of affairs regarding aging and pathology that sometimes renders their effects indistinguishable if indeed a pure and separable effect of age, *per se* exists. Alluding to an "age effect" here is merely a convenient way to summarize measurable changes which correlate with chronological age. Some theorists find it useful to view post maturational aging as a gradual process of diffuse brain damage.

A well known theory on the behavioral effects of aging holds that aging is normally accompanied by a reduction in neural excitability one manifestation of which is a loss of central inhibitory control (Birren, 1959) This hypothesis was derived from behavioral data indicating that older persons show a reduced capacity to withhold responses in laboratory performance tasks. Obviously such behaviors involve high levels of cognitive function while caloric nystagmus is simply reflexive. This ready application to diverse aging phenomena marks the appeal of the central inhibitory loss notion. A testable hypothesis therefrom is that middle-aged subjects should be less able to exert voluntary control over induced nystagmus than young adults. A related hypothesis is based on the phenomenon of suppression (Barber &

Zusammenfassung

293 untersuchte, schwindelanfällige Patienten im Alter zwischen 20 und 88 Jahren wurden der kalorischen-Nystagmus- und Békésy-Gehör Prüfung unterworfen. Bis in das 7. Lebensjahrzehnt konnte eine Reaktionszunahme mit dem Alter festgestellt werden, danach eine Abnahme. Dies konnte am besten an hand der Frequenz und der langsamen Nystagmuskomponente, weniger ausgeprägt an hand der Amplitude, der Latenz, der Dauer und der schnellen Nystagmuskomponente gezeigt werden. Altersabhängige Nystagmuszunahme war ausgeprägter im warmen Bereich als im kalten. Das Vorkommen von spontanem und statischem Nystagmus war nicht altersbedingt. Hörvermögen und kalorischer Nystagmus wiesen keine gegenseitige Abhängigkeit auf wenn gleiche Altersgruppen miteinander verglichen wurden, mit einer Ausnahme: Männliche Patienten mit starkem Gehörverlust im hohen Frequenzbereich wiesen einen stärkeren Nystagmus auf als jene ohne Gehörverlust, ganz gleich welchen Alters, wobei man an einen häufig an Cochlea und Vestibularapparat auftretenden Degenerationsprozess denken kann. Eine spekulative Erklärung der beobachteten Alterserscheinungen wurde auf der Basis vaskulärer Einflüsse und Verlust zentraler Inhibition geboten.

Résumé

Des examens d'ouïe — ceux de Békésy et ceux basés sur le nystagmo calorique — furent effectués avec 293 patients sélectionnés, souffrant de vertige (étourdissement) et dont l'âge variait entre 20 ans et 88 ans. Tous les paramètres de nystagme manifestaient une reactivité croissante au fur et à mesure que l'âge avançait dans la septième décade après quoi un déclin s'accentuait. La meilleure façon de le démontrer était par fréquence et vélocité de phase lente une démonstration moins accusée s'obtient par amplitude latence durée, et vélocité de phase rapide

Des augmentations de l'âge dans le nystagme etaient plus prononcées pour les stimulus chauds qu'elles ne l'étaient pour les stimulus froids. Le taux des nystagmes spontanés ou positionnels ne variait point avec l'âge des patients.

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Une explication spéculative des effets observés quant à l'âge fut formulée par nous, s'appuyant sur des influences vasculaires, ainsi que sur la *perte d'inhibition centrale*.

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Barber H. O. & Wright, G 1967 *Release of nystag-*

more severely occluded labyrinthine vessels, as would be expected in very old subjects the vasodilatory augmentation by warm calorics would become less and less effective. An additional factor in the very old subject is that the progressive depression of neural excitability will have become quite advanced and vestibular hypo-reactivity will come to the fore.

For the cold stimulus the vasoconstrictive action produces an opposite effect. It tends to reduce the resting discharge of the irrigated side allowing the discharge of the contralateral side to predominate. As is well known this produces nystagmus which beats away from the cold irrigated side In the young subject, the cold stimulus constricts his patent blood vessels effectively and he may respond with even longer durations and shorter latencies to cold water than he does to warm. On the already partially constricted vessels of middle aged subjects however we assume that the cold water produces little further constriction. One might expect the subsequent caloric response to be quite weak in middle-aged subjects, but a moderate nystagmus is still induced due to the relaxation of inhibitory control Apparently inhibitory release compensates for the deficient vasoconstrictive action so that middle aged subjects show about the same response strength to cold stimulation as the young.

In the foregoing, which admittedly is largely conjectural two augmenting mechanisms have been proposed as bases for the intensified nystagmus following warm irrigations in middle-aged subjects namely vasodilation of narrowed vessels and central inhibitory release. For cold irrigations inhibitory release alone facilitates nystagmus in middle-aged subjects despite a compromised vascular influence. The augmenting mechanisms appear to override, for two or three decades, an opposing force which likewise develops with age depressed neural excitability The latter is proposed to become more prominent in older age (> 70) as manifested by caloric hypo-reactivity

A quotation from Pavlov is most pertinent to the present conception the inhibitory process first and foremost and then the liveliness of the nerve processes, suffer from the development of senile changes." (Gakkel & Zinina 1953)

VI Summary

Caloric nystagmus and Békésy hearing tests were administered to 293 screened dizziness patients ranging in age from 20 to 88 All nystagmus parameters revealed increased responsivity with age into the 60 s followed by a decline. This was shown best by frequency and slow phase velocity and to a lesser extent by amplitude latency duration and fast phase velocity Age increments in nystagmus were more pronounced for warm stimuli than cold. The incidence of spontaneous or positional nystagmus did not vary with age. Hearing acuity and caloric nystagmus showed no intercorrelations when age was held constant with one exception Males with high frequency hearing losses manifested stronger nystagmus than subjects without loss regardless of age suggesting a decremental process common to the cochlea and vestibular apparatus. A speculative explanation of the observed age effects was offered based on vascular influences and loss of central inhibition.

Zusammenfassung

293 untersuchte, schwindelanfällige Patienten im Alter zwischen 20 und 88 Jahren wurden der kalorischen-Nystagmus- und Békésy-Gehör Prüfung unterworfen. Bis in das 7 Lebensjahrzehnt konnte eine Reaktionszunahme mit dem Alter festgestellt werden, danach eine Abnahme. Dies konnte am besten an hand der Frequenz und der langsamen Nystagmuskomponente, weniger ausgeprägt an hand der Amplitude, der Latenz, der Dauer und der schnellen Nystagmuskomponente gezeigt werden. Altersabhängige Nystagmuszunahme war ausgeprägter im warmen Bereich als im kalten. Das Vorkommen von spontanem und statischem Nystagmus war nicht altersbedingt. Hörvermögen und kalorischer Nystagmus wiesen keine gegenseitige Abhängigkeit auf, wenn gleiche Altersgruppen miteinander verglichen wurden, mit einer Ausnahme: Männliche Patienten mit starkem Gehörverlust im hohen Frequenzbereich wiesen einen stärkeren Nystagmus auf, als jene ohne Gehörverlust, ganz gleich welchen Alters, wobei man an einen häufig an Cochlea und Vestibularapparat auftretenden Degenerationsprozess denken kann. Eine spekulative Erklärung der beobachteten Alterserscheinungen wurde auf der Basis vaskulärer Einflüsse und Verlust zentraler Inhibition geboten.

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Some reports have indicated however that results from all of these special tests can, on occasion, be misleading. Brand & Rosenberg (1963) Johnson & House (1964) and Shapiro & Naunton (1967) have reported patients with cochlear audiologic signs who, in fact, had a retrocochlear neoplasm. Conversely Jerger et al. (1961) ha e reported that some patients with a sudden hearing loss will show audiologic signs indicating a retrocochlear lesion although neurologic studies are negative with respect to neoplasm.

It thus seemed worthwhile to attempt an evaluation of the contribution of audiology to localization of a lesion affecting the auditory system. The importance of a total work-up involving several disciplines has been documented in recent monographs and articles by Busis (1965) Eichel, Hedgecock & Williams (1966) House (1968) Parker Decker & Richards (1968) Stroud & Thalmann (1969) and in the Third Workship Proceedings (1969 a 1969 b) The special concern regarding the audiologic contribution stems from the well-known facts that hearing loss is often the patient s first complaint—with lesions involving the VIIIth nerve, for example—and that early detection is important. (Olivecrona, 1967 House, 1968)

The aims of the present survey were· (1) to assess the role of pure-tone findings and speech discrimination scores in localization of lesion (2) to report the accuracy of four special tests in localization of lesion, (3) to determine whether certain constellations of test results can help predict a particular kind or location of lesion and (4) to explore trends that may indicate directions for more definitive studies.

Subjects

Patients with sensorineural hearing loss, who had been referred for special audiologic testing to help n localization of lesion, were divided into groups as shown in Table 1 *Menière s Disease* (N 147) *Other Cochlear Lesion* (N-77) *Retroc chlear Lesion* (N-88) and *Sudden L s* (N 100)

Menière disease

The subjects in this group all had the symptom-triad of hearing loss, tinnitus, and episodic spinning" vertigo that defines Menière s disease, and the three symptoms were clearly interrelated. This triad of symptoms has been associated consistently with a hydrops of the labyrinth (Hallpike & Cairns, 1938 Lindsay 1942 Schuknecht, 1963 Altmann & Kornfeld, 1965 Lindsay Kohut & Scarra, 1967). All patients were diagnosed by otolaryngologists on the staff at the University of California, San Francisco, who were highly alert to the possibility of retrocochlear involvement. X-ray

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(*c*) cysts, (*d*) brain-stem gliomas, and (*e*) other Sheath tumors included those labeled neuroma, neurinoma, perineural fibroblastoma, neurilemmoma, schwannoma, and acoustic (VIIIth nerve) tumor. Among the cysts were *epidermoid*, *arachnoid*, and those referred as "type unknown." The brain-stem glioma classification included astrocytomas. Diagnoses are listed in Table 2. Insofar as could be determined from operative reports, the neoplasm was ipsilateral to the hearing impairment in all instances.

Sudden loss

All of these patients had had dramatic onset of hearing loss, usually severe. Descriptions of the onset were vivid and detailed, in contrast to those given by the other patients in the survey. Tinnitus, vestibular symptoms, or both had sometimes occurred simultaneously with the hearing loss. While the tinnitus, if occurring, tended to persist, the vestibular symptoms, although sometimes lasting for several days, typically failed to recur after subsiding. In short, the onset of sudden loss, with or without tinnitus and vestibular symptoms, was related to a single episode. Obscure etiology is inherent in the definition of sudden loss employed here. Loss of hearing due to head injury or mumps, for example (see Welsh & Welsh, 1963), is not included in the definition.

All patients in the *Sudden Loss* group were examined and diagnosed by staff otolaryngologists. Neurologic consultation and X-rays of the internal auditory meati and petrous pyramids (all normal) were obtained for most of these patients and several in the group were referred for complete medical and neurologic studies to rule out retrocochlear involvement. The literature suggests, however, that retrocochlear lesion in the form of a neoplasm is rarely found in hearing loss of sudden onset as defined above. Sheehy (1960) reported three patients with retrocochlear lesion in a series of 247 patients with sudden loss. Hallberg (1956) reported two in a series of 178 patients. None were found among our *Sudden Loss* patients. Of our 88 *Retrocochlear Lesion* patients, only one, a patient with a meningioma, reported a sudden onset of hearing loss. She described her loss as beginning with a "pop." However, she presented with other non-auditory symptoms of retrocochlear involvement.

Procedure

The audiologic records for all of these groups were obtained over a period of approximately 6 years. The tests were done when the patients were referred to us and then, at a later date, their records were checked for the final diagnosis. Although the patients forming the *Retrocochlear Lesion group* were unselected, a number of patients with Menière's disease and some who had experienced sudden loss were omitted because they had not been given the special tests. The special requirements already outlined for the *Other Cochlear Lesion* group served to exclude as many as 50 patients, most of whom had symptoms suggesting hydrops. Thus, the sampling, although believed to be unbiased, was not truly random; neither did it represent a critical incidence. At several points throughout the survey, analyses were undertaken on pertinent questions. The Ns for these analyses differed according to the number of patients on whom data were available at the time.

The audiologic evaluations were done in one clinic by audiologists employing essentially the same test methods. In the majority of cases the writer either did the evaluation or participated as an observer.

studies of the internal auditory meati (all normal) were obtained for 76 of the patients. Neurologic consultation was arranged for all patients when there was suspicion of retrocochlear lesion.

In two patients, the presence of the hearing loss-tinnitus-vertigo triad was deceptive. One patient was eventually found to have a cyst in the cerebellopontine angle and the other a glomus tumor invading the labyrinth. The first patient was included in the *Retrocochlear Lesion* group the second was excluded from the survey because involvement of the VIIIth nerve, although strongly suspected, could not be verified Golding Wood (1960) described a patient whose symptoms simulated Menière s disease despite the presence of an acoustic neurinoma but stressed the infrequency of such a case

Other cochlear lesion

In these patients, vestibular symptoms differed from the episodic, spinning vertigo of Menière s disease or were absent About one-third of the group had symptoms and signs suggesting hydrops often heard complaints, for example were roaring" tinnitus, fluctuating hearing loss, sensitivity to loud sounds, and a feeling of fullness. The most common diagnoses were atypical Menière s disease" and labyrinthine hydrops" The remaining two-thirds of the group had hearing losses thought to be associated with such factors as vascular disorders lues, allergy and diabetes. Most often the diagnosis was end-organ lesion, etiology unknown Patients with hearing loss primarily related to prolonged noise exposure ototoxic drugs or old age were excluded

All patients in the *Other Cochlear Lesion* group had X ray studies of the internal auditory meati and petrous pyramids, including tomograms in many instances. Over half of this group had also been examined by a neurologist. All neurologic studies and examinations were normal According to published observations (Crabtree & House, 1964 Valvassori 1969)

Table 1 *Patients who had been referred for special audiologic testing in localization of lesion*

Classification	*Number*
Menière s Disease	147
Other Cochlear Lesion	77
Retrocochlear Lesion	88
Sudden Loss	100

retrocochlear lesions are sometimes missed by radiography in the absence of iophendylate studies. Such studies were obtained on only a few patients in our group It can only be said that patients were excluded from the *Other Cochlear Lesion* group at the slightest suspicion of retrocochlear lesion expressed by the otolaryngologists, neurologists and neurosurgeons involved.

Retrocochlear lesion

Eighty three of the 88 patients in the *Retrocochlear Lesion* group had surgically identified neoplasms, four had multiple sclerosis, and one had an internal carotid aneurysm shown by vertebral arteriograms to be pressing against the VIIIth nerve in much the same manner as a tumor Classification of neoplasms was difficult because of different terminology used by individual neurologists, neurosurgeons, otolaryngologists, and histopathologists, but the following classifications seemed appropriate for this survey (*a*) sheath tumors, (*b*) meningiomas,

Table 2. *Diagnoses for the* Retrocochlear Lesion *group*

Diagnosis	*Number*
Sheath tumor	49
Meningioma	8
Cyst	11
Brain-stem glioma	6
Other[a]	14
	88

Includes multiple sclerosis (4), malignant tumor of the posterior fossa (2), cholesteotoma (2), chondroma (1), hemangioendothelioma (1), neurysm acting as a tumor (1), neurof broma with von Recklinghausen s disease (1), granulomatous meningitis (1), and hemangioblastoma (1).

(*c*) cysts, (*d*) brain-stem gliomas, and (*e*) other. Sheath tumors included those labeled neuroma, neurinoma, perineural fibroblastoma, neurilemmoma, schwannoma, and acoustic (VIIIth nerve) tumor. Among the cysts were epidermoid, arachnoid, and those referred as "type unknown." The brain-stem glioma classification included astrocytomas. Diagnoses are listed in Table 2. Insofar as could be determined from operative reports, the neoplasm was ipsilateral to the hearing impairment in all instances.

Sudden loss

All of these patients had had dramatic onset of hearing loss, usually severe. Descriptions of the onset were vivid and detailed, in contrast to those given by the other patients in the survey. Tinnitus, vestibular symptoms, or both had sometimes occurred simultaneously with the hearing loss. While the tinnitus, if occurring, tended to persist, the vestibular symptoms, although sometimes lasting for several days, typically failed to recur after subsiding. In short, the onset of sudden loss, with or without tinnitus and vestibular symptoms, was related to a single episode. Obscure etiology is inherent in the definition of sudden loss employed here. Loss of hearing due to head injury or mumps, for example (see Welsh & Welsh, 1963), is not included in the definition.

All patients in the *Sudden Loss* group were examined and diagnosed by staff otolaryngologists. Neurologic consultation and X-rays of the internal auditory meati and petrous pyramids (all normal) were obtained for most of these patients and several in the group were referred for complete medical and neurologic studies to rule out retrocochlear involvement. The literature suggests, however, that retrocochlear lesion in the form of a neoplasm is rarely found in hearing loss of sudden onset as defined above. Sheehy (1960) reported three patients with retrocochlear lesion in a series of 247 patients with sudden loss. Hallberg (1956) reported two in a series of 178 patients. None were found among our *Sudden Loss* patients. Of our 88 *Retrocochlear Lesion* patients, only one, a patient with a meningioma, reported a sudden onset of hearing loss. She described her loss as beginning with a "pop." However she presented with other non-auditory symptoms of retrocochlear involvement.

Procedure

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The audiologic evaluations were done in one clinic by audiologists employing essentially the same test methods. In the majority of cases the writer either did the evaluation or participated as an observer.

Pure-Tone Findings

Bilateral pure-tone loss

The first consideration regarding pure-tone findings was the occurrence of bilateral pure tone loss (see Table 3) defined arbitrarily by threshold levels of greater than 25 dB in both ears at four or more of the six octave interval frequencies within the 250–8 000 Hz range

In the *Menière s Disease* group bilateral loss occurred in 26 of the 147 patients Their histories suggested that, in seven of the 26 both ears were affected at about the same time in nine the second ear was affected at a later date and in the remaining 10 the information regarding onset was unreliable In two of the last 10 it seemed likely that the impairment in the poorer ear was superimposed on a bilateral presbycusis Actually then 24 (16%) patients in the *Menière s Disease* group had bilateral loss stemming from a presumed hydrops.

Of the 77 patients in the *Other Cochlear Lesion* group 15 showed bilateral loss but in only nine (12%) were both ears seemingly affected by the same kind of impairment The onset was at about the same time for both ears in seven of the nine, while in the other two the history of onset was vague In the remaining six of the 15 with bilateral loss, hearing in one ear was noticeably poorer than in the other and the impairment in the poorer ear seemed to have been superimposed on a previous bilateral impairment of a different kind—from presbycusis in three and from long time noise exposure in three

Of the 88 patients in the *Retrocochlear Lesion* group four showed bilateral pure tone impairment. In only one, a patient with von Recklinghausen s disease, did the loss in both ears stem from the same kind of impairment. In the remaining three hearing in one ear was noticeably poorer than in the other and the impairment in the poorer ear seemed to have been superimposed upon a previous bilateral impairment—from presbycusis in two and from noise exposure in one.

In the *Sudden Loss* group bilateral involvement was present in 18 of the 100 patients. In four of the 18 both ears seemingly were affected at the same time in two of these four the onset seemed associated with a major operation In the remaining 14 of the 18 patients the history revealed that at the time of sudden onset in one ear a long-standing loss had been present in the other ear In these 14 patients the opposite-ear loss reportedly had not been of sudden onset and its etiology was indeterminable

It should be stressed that we are talking here only of threshold impairment in four of the *Retrocochlear Lesion* patients to be discussed later bilateral impairment that had not been reflected by pure tone threshold elevations could be demonstrated by tone decay and Bekesy tracings Also we are excluding patients in whom a tumor extended to the opposite side at a late stage

Bilateral pure-tone loss from the same impairment was thus uncommon in all groups. It was highest in the *Menière s Disease* and *Other Cochlear Lesion* groups (16% and 12% respectively) relatively less common in the *Sudden Loss group* (4%) and uncommon in the *Retrocochlear Lesion* group (1 2%) Thus if von Recklinghausen s disease is excluded from consideration retrocochlear lesion would rarely be expected with bilateral pure-tone loss.

The literature presents the same general trends for bilateral loss in patients with Menière s disease sudden loss, and retrocochlear involvement although widely varying percentages have been given probably in part because of differences in definition For patients with Menière s disease, bilateral loss was reported by Hallberg (1956) in 19 of 56 patients (34%) and by Goldingwood (1960) in 94 of 214 patients (44%) In contrast Day (1963) reported bilateral involvement in only 5 to

10 % of Menière's disease patients, with the two ears usually becoming involved simultaneously. Delayed involvement (after 6 months) in the second ear occurred in less than 2% of his more than 1 500 cases" Schuknecht (1963) reported that about 10% of patients with Menière's disease had bilateral loss.

Regarding retrocochlear impairment, Nager (1969) stated that "Bilateral eighth nerve tumors occur in about 4% of all acoustic neurinomas. In most instances they are associated with von Recklinghausen's disease." Hitselberger & Hughes (1968) recently reported bilateral involvement in 12 of 212 patients with VIIIth nerve tumor. Without mentioning von Recklinghausen's disease, they attributed these tumors to "an abortive form of neurofibromatosis". Caparosa (1966) presented three patients with bilateral VIIIth-nerve involvement, all three of whom had von Recklinghausen's disease. On the other hand, von Recklinghausen's disease was evident in only one of the two patients with bilateral acoustic tumors recently reported by DeMoura et al. (1969).

Although bilateral hearing losses have been reported with brain-stem lesions, in contrast to the above VIIIth-nerve lesions, such data are sparse. Bilateral reduction in hearing sensitivity was described by Horrax & Bailey (1925) in five of 12 patients with pineal body tumors (pinealoma); several years later, however, Nylen (1939) concluded that hearing reduction was not a common finding with pinealoma, and that it was a late symptom when it did appear. In other kinds of brain-stem lesion Nylen reported very low percentages of bilateral hearing loss.

With respect to sudden loss, Hallberg (1956) found only two patients with bilateral involvement in a series of 89 patients, while Schuknecht et al. (196-) stated that both ears were involved in less than 70 of such patients.

Total unilateral loss

Total unilateral loss was defined by an inability to hear pure tones in the affected ear at the audiometer limits (90 dB at 250 and 8 000 Hz, and 110 dB at the intervening octave-interval frequencies re audiometric zero). Included in this definition are patients who responded near audiometer limits for the frequencies 250 and 500 Hz, but who were not sure whether they heard an actual tone or simply *felt* the signals generated in the earphone.

As shown in Table 3, total unilateral hearing loss was found in one of the *Menière's Disease* group, in none of the *Other Cochlear Lesion* group, in six of the *Retrocochlear Lesion* group (7%), and in nine of the *Sudden Loss* group (9%).[1]

Goodman (1965), employing the same definition of total loss, found only one patient with total unilateral loss in his group of 43 patients with Menière's disease and pointed up the rarity of such loss by describing this patient at length. Hallberg (1956) reported five patients with total unilateral loss in a series of 56 patients with Menière's disease. He also reported that, in a series 89 patients with sudden unilateral loss, the deafness was total in 36%. In neither his Menière's disease group nor his sudden loss group was a definition of total unilateral loss given. Brand & Rosenberg (1963) reported 10 cases of total unilateral loss in a selected series of 22 patients with VIIIth-nerve tumor, and Johnson (1965) reported 20 in a series of 110 such patients (18%). Buey & Isamat (1961) stated that acoustic neurinoma "... is characterized by progressive deafness which soon reaches the point where all useful hearing is lost"

According to evidence from the literature and from the present series, then, total unilateral loss is found primarily in patients experiencing sudden loss or loss associated with VIIIth-nerve tumor. It seems unlikely that our nine *Sudden Loss* patients with complete unilateral loss had an VIIIth-nerve tumor. In one, hearing returned almost completely and in

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Table 5 *Ages of patients with better-ear high-tone loss*

Group	Better ear	Mean age	Number
Menière's Disease	Normal	43	48
	High-tone loss	54	39
Other Cochlear Lesion	Normal	42	26
	High-tone loss	55	22
Retrocochlear Lesion	Normal	35	41
	High-tone loss	58	16
Sudden loss	Normal	41	40
	High-tone loss	59	17

causal relationship. Further he found that in approximately 50 of 100 patients with Menière's disease, a bilateral high-tone loss was present before the onset of hydrops in the affected ear. He felt that in many older persons the hydrops was thus superimposed on an existing loss. Direct evidence in this regard was precluded in the present survey because the patients were seen for the first time only after the loss in the affected ear had occurred. In a sample composed of the first 87 *Menière's Disease* patients, however, the mean age of 39 patients with better-ear high-tone loss was 54 years and the mean age of 48 patients with normal hearing in the better ear was 43 years. The difference of 11 years was significant at the 0.1 level of confidence. Thus, age did appear to play a role in the high-tone loss of the better ear. Considering, then, that hearing loss due to aging is usually bilateral, it does seem likely that high-tone loss was present in the affected ear before the onset of hydrops. The question of causal relationship, however, is beyond the scope of this survey. Ages of other patients who were part of the survey at the same time as the 87 *Menière's Disease* patients were also tallied, and the data are shown in Table 3. In all groups, better-ear high-tone loss seemed associated with older age.

A few of the *Menière's Disease* patients, not included in Table 5, showed a better-ear high-tone loss that seemed entirely due to noise exposure. Because hearing loss from long-time exposure to noise is usually bilateral, although not necessarily the same in each ear, it appears likely that in these patients too high-tone loss existed in the affected ear prior to the onset of hydrops. In any event, because of the prevalence of high-tone loss in the better ear of all groups, it seemed incumbent to include both ears in the classification of pure-tone patterns. The purpose was to permit a more accurate delineation of any presumably superimposed loss in the affected ear.

Accordingly, in cases of better-ear high-tone loss, a pre-existing, bilaterally equal, high-tone loss was assumed and the poorer-ear impairment was viewed as a subsequent superimposition. Looking at both ears, then, five classes of pure-tone patterns emerged for the frequency range of 250 to 8 000 Hz: (1) patterns in which the configurations for the two ears were essentially parallel, (2) patterns in which the two configurations closed or almost closed at the high end of the frequency range, (3) patterns that showed areas or points of constriction between the two configurations, (4) patterns in which a widening occurred between the two configurations in the high-tone range, and (5) patterns in which the two configurations closed or almost closed at both ends of the frequency range, widening in the middle.

Patterns that were highly similar in shape and fairly equal with respect to differences between the ears were sorted out and combined in a representative schematic. A number of such schematics resulted for each group, as shown in Fig. 1 through 5. When the better ear was normal, a straight line 10-dB level was employed as a reference; when the better ear showed a high-tone loss, schematics believed to be representative of the most frequently occurring slopes in each group served as references. In grouping the configurations for the schematics, a special effort was made to portray the actual differences in threshold levels between the two ears.

In defining these patterns, the statistician's "best-fitting straigth line" through the thresholds for each ear from 250 to 8 000 Hz was

Table 3 Bilateral losses and total unilateral losses among the groups studied

Groups	Initial number	Bi lateral loss	Total unilateral loss	Re-maining number
Menière s Disease	147	26	1	120
Other Cochlear Lesion	77	15[a]	0	62
Retrocochlear Lesion	88	4[a]	6	76[b]
Sudden Loss	100	18[a]	9	73

[a] In only 24 *Menière's Disease* patients (16%) nine *Other Cochlear Lesion* patients (12%) one *Retrocochlear Lesion* patient (1.2 %) and four *Sudden Loss* patients (4%) did the loss in each ear seem due to the same kind of impairment.
[b] See text for explanation of the mathematical discrepancy

another it returned partially In two the loss had been present for many years without neurologic signs. In another the loss seemed clearly associated with a virus, and in yet another the loss occurred in an operated ear several weeks after a stapedectomy Of the remaining three, one had had an extensive neurologic work up in which all findings were normal, while in the other two the examining otologist felt confident in ruling out an VIIIth-nerve tumor on the basis of the otologic examination and the circumstances relating to the onset. In any case the evidence suggests that a total unilateral loss in the absence of a history of sudden hearing loss should arouse suspicion of retrocochlear involvement specifically involvement of the VIIIth nerve

Pure-tone patterns

Patients with bilateral loss and patients with total unilateral loss were excluded from the remainder of the survey Those remaining for analysis of pure tone patterns (see Table 3) were as follows.
Menière s Disease (120) *Other Cochlear Lesion* (62) *Retrocochlear Lesion* (76) and *Sudden Loss* (73)

The number remaining in the *Retrocochlear Lesion* group requires explanation. Three of the four patients with bilateral loss (that is, all except the one with von Recklinghausen s disease) were retained because the better-ear impairment was marginal and involved primarily the high tones. As stated previously the better ear impairment in these three patients probably stemmed from exposure to noise (in one) and old age. Five patients were omitted because their pure-tone patterns could not be classified easily according to our system They will be discussed in a later section. If from the initial number of 88 we subtract these five along with the six patients with total unilateral loss and the one patient with von Recklinghausen s disease and bilateral loss, we are left with 76.

The occurrence of high-tone loss in the better ear of a number of patients complicated the classification of pure-tone patterns. High-tone loss was defined by threshold levels no greater than 25 dB for frequencies 250 through 1 000 Hz accompanied by levels greater than 25 dB at 2 000 4 000 and 8 000 Hz, at 4 000 and 8 000 Hz or at 8 000 Hz only A dip at 4 000 Hz was not counted as a high tone loss. Defined in this manner high-tone loss occurred in 55 of the 120 *Menière s Disease* patients (46%) in 31 of the 62 *Other Cochlear Lesion* patients (50%) in 25 of the 76 *Retrocochlear Lesion* patients (33%) and in 26 of the 73 *Sudden Loss* patients (36%) (see Table 4)

Lindsay (1960) thought that high tone loss in the better ear of patients with Menière s disease occurred often enough to suggest a

Table 4 *High-tone loss in the better ear*

Group	Total number	Number with better-ear high-tone loss
Menière s Disease	120	55 (46)
Other Cochlear Lesion	62	31 (50)
Retrocochlear Lesion	76	25 (33)
Sudden Loss	73	6 (36)

Defined by threshold levels no greater than 25 dB for frequencies 250 through 1 000 Hz accompanied by levels greater than 25 dB at 2 000, 4 000, and 8 000 Hz, at 4 000 and 8 000 Hz, or at 8 000 Hz only

Table 5. *Ages of patients with better-ear high-tone loss*

Group	Better ear	Mean age	Number
Menière's Disease	Normal	43	48
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Sudden loss	Normal	41	40
	High-tone loss	59	17

causal relationship Further he found that in approximately 50 of 100 patients with Menière s disease a bilateral high-tone loss was present before the onset of hydrops in the affected ear He felt that in many older persons the hydrops was thus *superimposed* on an existing loss. Direct evidence in this regard was precluded in the present survey because the patients were seen for the first time only after the loss in the affected ear had occurred. In a sample composed of the first 87 *Menière's Disease* patients, however the mean age of 39 patients with better-ear high-tone loss was 54 years and the mean age of 48 patients with normal hearing in the better ear was 43 years The difference of 11 years was significant at the 0 1 level of confidence. Thus, age did appear to play a role in the high-tone loss of the better ear Considering, then, that hearing loss due to aging is usually bilateral, it does seem likely that high-tone loss was present in the affected ear before the onset of hydrops. The question of causal relationship, however is beyond the scope of this survey Ages of other patients who were part of the survey at the same time as the 87 *Menière s Disease* patients were also tallied, and the data are shown in Table 5 In all groups, better-ear high-tone loss seemed associated with older age.

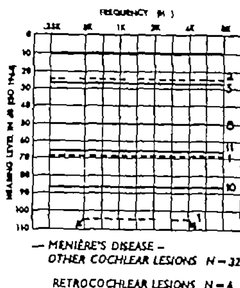
A few of the *Menière s Disease* patients, not included in Table 5 showed a better-ear high-tone loss that seemed entirely due to noise exposure. Because hearing loss *from long-time* exposure to noise is usually *bilateral*, although not necessarily the same in each ear it appears likely that in these patients too high-tone loss existed in the affected ear prior to the onset of hydrops. In any event, because of the prevalence of high-tone loss in the better ear of all groups, it seemed incumbent to include both ears in the classification of pure-tone patterns. The purpose was to permit a *more* accurate delineation of any presumably *superimposed loss in* the affected ear

Accordingly in cases of better-ear high-tone loss, a pre-existing, bilaterally equal, high-tone loss was assumed and the poorer-ear impairment was viewed *as a subsequent super* imposition. Looking at both ears, then, five classes of pure-tone patterns emerged for the frequency range of 250 to 8 000 Hz: (1) patterns in which the configurations for the two ears were essentially parallel (2) patterns in which the two configurations closed or almost closed at the high end of the *frequency range* (3) patterns that showed areas or points of constriction between the two configurations (4) patterns in which a widening occurred between the two configurations in the high-tone range; and (5) patterns in which the two configurations closed or almost closed at both ends of the frequency range, widening in the middle.

Patterns that were highly similar in shape and fairly equal with respect to differences between the ears were sorted out and combined in a representative schematic. A number of such schematics resulted *for each group as* shown in Fig. 1 *through 5 When the* better ear was *normal*, a straight line 10-dB level was employed as a reference when the better ear showed a high-tone loss, schematics believed to be representative of the most frequently occurring slopes in each *group* served as references. In grouping the *configurations for the schematics, a special effort* was made to portray *the actual differences in threshold* levels between the two ears.

In defining these patterns, the statistician s best-fitting straight line" through the thresholds for each ear from 250 to 8 000 Hz was

a



b

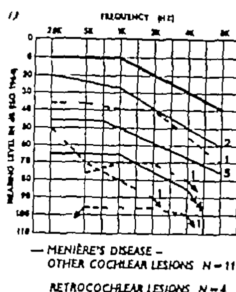


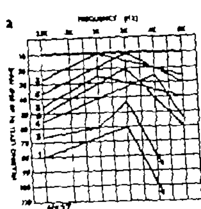
Fig 1 (*a*) A comparison of parallel" patterns for the combined *Menière's Disease + Other Cochlear Lesion* group and for the *Retrocochlear Lesion* group. Normal hearing in the better ear (*b*) A comparison of parallel patterns for the combined *Menière's Disease + Other Cochlear Lesion* group and for the *Retrocochlear Lesion* group. High-tone loss in the better ear

employed two straight edges, one for each ear served in place of actual lines If the two straight edges parted no more than 10 dB at either end of the frequency range relative to the parting at the other end the pattern of configurations was classed as parallel" If the two straight edges parted more than 10 dB at 250 Hz compared to the parting at 8 000 Hz, the pattern of configurations was classed as "closing" When the two edges parted more than 10 dB at 8 000 Hz compared to the parting at 250 Hz, the pattern was classified as widening" The constricting" patterns were typically associated with rising–falling configurations in the affected ear and were easily classified by inspection Similarly the closing both-ends" patterns typically resulted from trough-shaped configurations in the affected ear and were also easily classified by inspection which, as a matter of fact, was true in most cases for all of the patterns.

Arbitrary decisions were required for a few patterns that were difficult to classify For example when no response occurred at 8 000 Hz in the affected ear pattern classification rested on the relation between the two configurations from 250 to 4 000 Hz, unless a response to 8 000 Hz at audiometer limits would have resulted in a widening' classification. When no response occurred at either 8 000 Hz or 4 000 Hz in the affected ear the classification was based on the 250- to 2 000-Hz range unless a response to 4 000 Hz at audiometer limits would have resulted in a widening" classification. When no response occurred in the affected ear at 250 Hz, the classification was based on the pattern of configurations within the 500- to 8 000-Hz range. In cases of a notch at 4 000 Hz in the better ear a pre existing bilateral notch was assumed In three instances of better-ear conductive loss, the bone-conduction hearing levels constituted the configuration for the unaffected ear necessitating the use of only the 250- to 4 000-Hz range.

Finally in five instances where a sharp widening followed a constriction the patterns were classified by inspection according to whether the constricting or the widening aspect prevailed

Table 6 summarizes the patterns for a comparison between groups. We may observe that the parallel" patterns were found in all groups, but in a noticeably smaller proportion of the



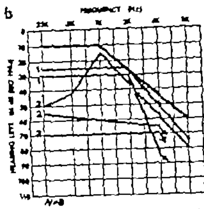


Fig. 2. (*a*) Examples of "constricting" patterns for the *Menière' Disease + Other Cochlear Lesion* combined group. Normal hearing in the better ear. (*b*) Examples of "constricting" patterns for the *Menière's Disease + Other Cochlear Lesion* combined group. High-tone loss in the better ear.

Retrocochlear Lesion group. A comparison of the parallel patterns found among the *Retrocochlear Lesion* group and the combined *Menière's Disease + Other Cochlear Lesion* group is shown in Fig. 1. Particularly of note are three patients in the *Retrocochlear Lesion* group who showed "parallel" patterns accompanied by a minimal difference between the ears on the order of 10–25 dB. In two of the three, hearing in the poorer ear was still within normal range. Of the three patients, two had a sheath tumor and one a brain-stem glioma. This minimal difference with parralled patterns occurred infrequently (five of 182 patients) in the combined *Menière's Disease + Other Cochlear Lesion* group and never in the *Sudden Loss group*. One other *Retrocochlear Lesion* patient showed a minimal-difference "parallel" pattern before developing a high-tone loss in the affected ear, which led to his placement in the "widening" group. This patient was described by Flower & Viehweg in 1961. Similar patterns accompanying retrocochlear involvement have appeared in other studies, but were not stressed by the investigators. For example, Goetzinger & Angell (1965) in presenting a series of audiograms for patients with retrocochlear lesion, included those of two patients in which the ear ipsilateral to a cerebellopontine-angle mass showed thresholds about 70 dB below those of the better ear across the frequency range. In any event, the presence of a minimal-difference parallel pattern should provide an immediate clue to the possibility of retrocochlear involvement.

"Constricting" patterns occurred almost exclusively in the *Menière's Disease* and *Other Cochlear Lesion* groups (see Table 6 and Fig. 2). It thus appears that these patterns may be a unique reflection of hydrops.

"Closing" patterns were predominant in the *Menière's Disease* and *Other Cochlear Lesion* groups (see Table 6 and Fig. 3), particularly in patients with better-ear high-tone loss. These patterns occurred only occasionally in the *Retrocochlear Lesion* group. Consequently, it seems that hydrops might be suspected in the *Other Cochlear Lesion* patients with "closing" patterns. Although these patterns occurred frequently in the *Sudden Loss* group, suspicion of hydrops is probably not justified. For one reason, the "closing" patterns of this group as well as those few that occurred for the *Retrocochlear Lesion* group typically accompanied a greater degree of loss compared to those for the *Menière's Disease* and *Other Cochlear Lesion* patients. For another reason, histopathologic evidence (Perlman & Kimura, 1959; Schuknecht et al., 1962) suggests that hydrops is not commonly found with sudden loss.

The "widening" patterns, although characteristic of the *Retrocochlear Lesion* group, oc

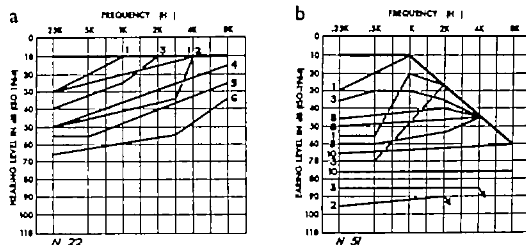


Fig 3 (*a*) Examples of closing patterns, *Menière's Disease + Other Cochlear Lesion* combined group. Normal hearing in the better ear (*b*) Examples of closing patterns, *Menière s Disease + Other Cochlear Lesion* combined group. High-tone loss in the better ear

curred frequently in the *Sudden Loss* group they occurred markedly less often in the *Menière s Disease* and *Other Cochlear Lesion* groups The probability of a widening' pattern in the *Retrocochlear Lesion* group was 0 67 compared with a probability of 0 13 in the combined *Menière s Disease + Other Cochlear Lesion* group In the absence of a history of sudden loss, then, suspicion of retrocochlear involvement should be aroused by a widening" pattern.

Examples of widening" patterns in the *Retrocochlear Lesion* group are shown in Fig. 4 Although the precipitously widening patterns (Fig. 4 *a*) are found frequently in the *Sudden Loss* group as well as in the *Retrocochlear Lesion* group the gradually widening patterns (Fig. 4 *b*) are unusual among the *Sudden Loss* group

In the case of widening" patterns, just as in the case of parallel" patterns, importance should be attached to minimal threshold differences between the ears. Two patients—one with a brain-stem glioma and the other with a chondroma—showed a widening" pattern of this kind (Fig. 4 *b*) which was seldom seen in the other groups. Housa, Gale & Hughes (1968) described five patients who had small

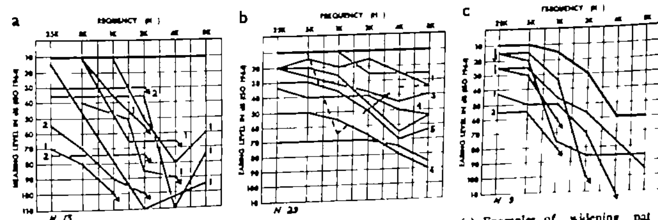


Fig 4 Examples of widening patterns (precipitous), *Retrocochlear Lesion* group. Normal hearing in the better ear (*b*) Examples of "widening patterns (gradual), *Retrocochlear Lesion* group. Normal hearing in the better ear ( ) Examples of widening patterns, *Retrocochlear Lesion* group. High-tone loss in the better ear

Table 6. *Summary of pure-tone patterns*

Figures in parentheses are percentages

Patterns	Menière's Disease	Other Cochlear	Retro-cochlear	Sudden Loss
Parallel	30 (25.0)	13 (20.9)	8 (11.0)	14 (19.2)
Constricting	25 (20.9)	10 (16.1)	1 (1.0)	1 (1.3)
Closing	52 (43.3)	21 (33.8)	10 (13.0)	28 (38.4)
Widening	11 (9.2)	12 (19.5)	51 (67.0)	29 (39.8)
Closing, both ends	2 (1.6)	6 (9 7)	6 (8.0)	1 (1.3)
Total	120	62	76	73

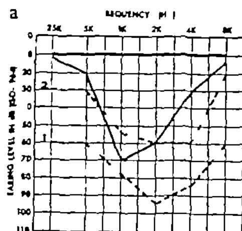
VIIIth-nerve tumors entirely within the internal auditory meatus. All five were said to have a high-tone loss in the affected ear although only one showed significant findings on special tests. Their audiograms were not presented. In any event, the evidence suggests that a widening" pattern with a minimal pure-tone threshold difference between the ears may be an early clue to the presence of a retrocochlear lesion. However because unilateral high-tone loss is a fairly common occurrence—for example, with various kinds of noise exposure and with long-standing loss from childhood—the audiologist must be extremely chary in attributing it to retrocochlear involvement.

Finally closing-both-ends" patterns were confined primarily to the *Other Cochlear Lesion* and *Retrocochlear Lesion* groups (Table 6 and Fig. 5), and were infrequent even there. Although they occurred with about equal probability in the two groups, they nevertheless should alert the audiologist to possible retrocochlear involvement.

It seems appropriate here to consider the groups individually and relate the pure-tone findings to those reported in the literature. For the *Menière's Disease* group, low-tone involvement (250 and 500 Hz) prevailed. Only in the few instances of widening and closing-both-ends patterns were the low tones less involved than the high tones. These results agree with the observations of Ophelm & Flottorp (1957), Lindsay (1960) and Schuknecht (1963) that low-tone loss is a *predominant* feature in Menière's disease. At the same time, there is no essential disagreement with the statement of Hallpike & Hood (1959) that in Menière's disease there may be high-tone loss, low-tone loss, or loss throughout the frequency range. Nor is there any contradiction to Goodman's (1965) observation of a great number of sharp peaks and dips in the affected ears. Peaks and dips were particularly noticeable in the constricting patterns.

Regarding the *Other Cochlear Lesion* group, the similarity of their patterns to the patterns of the *Menière's Disease* group doubtless reflects hydrops in many of the patients despite absence of the episodic vertigo required for a diagnosis of Menière's disease. Williams et al., (1950) and Lindsay & Schulthess (1858) have described patients with low-tone loss and certain other characteristics that should permit the diagnosis of endolympatic hydrops in the absence of vertigo. Day (1963) stated that the cochlear symptoms usually precede the vestibular symptoms in Menière's disease and suggested that treatment should begin with the *first cochlear symptoms in order to reverse the* pure-tone loss and to prevent vestibular symptoms from developing.

For the *Retrocochlear Lesion* group, the widening" patterns clearly predominated. Because high-tone involvement shown by the widening patterns is usually synonymous with falling configurations for the affected ear realistic comparisons with other studies can be made. Goodman (1957) found most configurations to be either flat or falling in a series of 18 patients with VIIIth-nerve lesion. Dix & Hallpike (1960) depicted the *pure-tone* configurations of 95 patients with VIIIth-nerve lesion. most configurations were falling; five were rising; several were flat, and some were not easily classifiable. Parker et al. (1968) in a series of 49 patients with retrocochlear lesion, found no characteristic shape to the curve, but some tendency for greater loss in the higher frequencies Johnson (1968) reported that, of 168 patients with acoustic neuromas, the pure-tone configurations revealed a high-



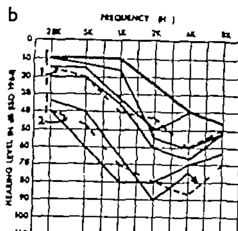


Fig 5 Examples of closing both-ends" patterns, *Othe Cochlear Lesion* and *Retrocochlear Lesion* groups. Normal hearing in the better ear (*b*) Examples of closing both-ends patterns, *Other Cochlear Lesion* and *Retrocochlear Lesion* groups.

tone loss in 64% a flat loss in 20% a low tone loss in 8% and a trough-shaped loss in the remaining 8% Accordingly the predominance of widening" patterns for our *Retrocochlear Lesion* group finds support in these four studies.

Two major patterns emerged for the *Sudden Loss* group one involving primarily the high tone range and the other involving the low tone range Comparisons with other surveys flounder here because of differences in portrayal and interpretation Hallberg (1956) Sheehy (1960) and Bosatra & DeStefan (1961) seem to agree however that hearing impairment in sudden loss may occur in any part of the frequency range

In summarizing the pure-tone findings it may be said that they are of definite value as alerting signs in the localization of lesion. In the absence of von Recklinghausen s disease, cochlear lesion is suggested with bilateral pure tone loss. In unilateral pure tone loss cochlear lesion is suggested with constricting" and closing" patterns. Constricting" patterns seem unique to hydrops of the labyrinth. Closing patterns suggest hydrops except in cases of sudden loss

In the absence of a history of sudden loss, *retrocochlear involvement is suggested* by total unilateral loss and by a widening" pure tone pattern. Retrocochlear involvement is also suggested by a closing both ends pattern and by a parallel pattern with a minimal difference between the ears (on the order of 10 to 25 dB)

Speech Discrimination Scores

In general speech discrimination scores of patients with Menière s disease worsen as hearing loss for pure tones becomes more severe. Goodman (1965) using the Rush–Hughes recordings of the Harvard PB50 lists, found a Pearson product moment correlation of 0 84 between the discrimination loss and the pure tone average for 42 patients with Menière s disease In the present survey for discrimination losses on the CID W 22 lists and Fletcherian pure-tone

verages, Pearson product-moment correlations of 0.74 and 0.77 were obtained in samples comprising the first 72 *Menière's Disease* patients and the first 60 *Other Cochlear Lesion* patients.

For the *Retrocochlear Lesion* group, scatter diagrams showed an obvious lack of correlation between the two measures. For the *Sudden Loss* group, meaningful correlations were precluded by a high proportion of 0% scores (which skewed the score distribution) and by a predominance of severe losses (which skewed the hearing level distribution).

The speech discrimination scores were obtained by monitored live voice presentation at a comfortable loudness level for the patient, usually 40 dB above his speech reception threshold, except in cases of a tolerance problem. The predictive value of the scores was reduced somewhat by the use of live-voice presentation, and we had to keep in mind a conclusion by Goodman (1965) that "In the temporal course of Menière's disease, hearing level for tones and discrimination loss for speech vary independently of one another in a significant proportion of cases". Nevertheless, our test score data seemed of interest, especially because our report includes patients with cochlear lesions as well as patients with retrocochlear lesions, in contrast to other reports that have dealt with one group or the other.

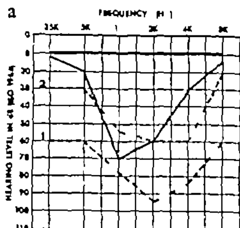
The procedure was first to inspect scatter diagrams of W 22 test scores versus the Fletcherian pure-tone averages. This was done for the combined *Menière's Disease* and *Other Cochlear Lesion* groups (N 137) employed in the above Pearson product-moment correlations and for 60 *Retrocochlear Lesion* patients who had already taken the speech discrimination test at the time these correlations were obtained. A clear dichotomy between the scores of the *Retrocochlear Lesion* group and the scores of the combined *Menière's Disease + Other Cochlear Lesion* group emerged at an average pure-tone level of approximately 50 dB—the amount of overlap between the scores of the two groups increasing progressively as threshold level increased. Accordingly, for purposes of prediction, attention was focused on scores at the lower levels (better thresholds) as shown in Fig. 6.

It may be seen that when W 22 scores below 48% occur for patients with Fletcherian averages less than 40 dB, retrocochlear involvement can be predicted with high confidence. Since the two patients in the *Menière's Disease + Other Cochlear Lesion* group who scored between 32% and 38% within the 40–43 dB level were 75 and 65 years old, the possibility of exceptional cases among the elderly should be entertained. Other exceptions might be patients with sharply-falling configurations through the speech range—for example, slopes 20 dB or greater per octave from 500 to 4 000 Hz. Two patients in the *Other Cochlear Lesion* group were omitted from Fig. 6 because of such extreme slopes beginning at 500 Hz. Fig. 6 shows, incidentally, that a high discrimination score at any of these levels does not necessarily rule out retrocochlear involvement.

The above observations were conservative for the reasons already mentioned, and the wise audiologist will doubtless exercise judgment in individual instances. For example, with Fletcherian averages under 24 dB and fairly flat configurations through 2 000 Hz, he may suspect retrocochlear involvement with W 22 scores as high as 80%, or perhaps even a few points higher, when the opposite ear shows a score within a few points of 100%. Also, he may suspect retrocochlear involvement with scores close to 0%, except in cases of severe hearing loss and provided that there is no history of sudden onset. For example, of all 182 patients in the combined *Menière's Disease* and *Other Cochlear Lesion* groups for whom special tests were done, only two scored 0%. Of these, one had a Fletcherian average of 60 dB, and the other 80 dB. Altogether only 14 of the 182 scored below 20%; the Fletcherian averages for these 14 ranged from

The Fletcherian average refers to the average of the best two of the three speech frequency thresholds—500, 1 000, and 2 000 Hz.

a



b

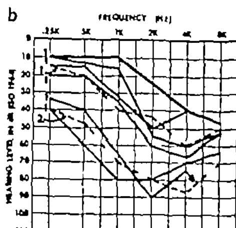


Fig 5 Examples of closing-both-ends patterns, *Other Cochlear Lesion* and *Retrocochlear Lesion* groups. Normal hearing in the better ear (*b*) Examples of closing-both-ends patterns, *Other Cochlear Lesion* and *Retrocochlear Lesion* groups.

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In the absence of a history of sudden loss, retrocochlear involvement is suggested by total unilateral loss and by a widening" pure-tone pattern. Retrocochlear involvement is also suggested by a closing both ends" pattern, and by a parallel pattern with a minimal difference between the ears (on the order of 10 to 25 dB)

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Alternate binaural loudness balance (ABLB)

The ABLB test for loudness recruitment was administered by presenting short-duration (approximately one second) tones of the same frequency alternately from one ear to the other with approximately a one-second interval between presentations. The frequencies selected were those showing a normal threshold for one ear (within 25 dB of audiometric zero) and a difference of at least 20 dB between the thresholds for the two ears.

For approximately 70% of the patients, the Fowler (1950) technique was followed, in which the tone of fixed intensity was presented to the better ear. At each of several successive 20-dB intensity levels above the threshold in the better ear several pairings of the tone were given. The patient reported verbally for each pairing whether the tone in the poorer ear was louder softer or the same in loudness as the tone in the better ear. The examiner through a bracketing procedure, found the level in the poorer ear at which the tone was judged to be equal in loudness to the tone in the better ear. When this procedure had been completed at several successive 20-dB levels in the better ear a laddergram was drawn which indicated the presence or absence of recruitment. Examples are shown in Fig. 7.

For 30% of the patients, the tone of fixed intensity was presented to the poorer ear (Jerger & Harford, 1960; Jerger 1962). A tone at a level 20 dB above the poorer-ear threshold was paired with tones at varying levels above the threshold for the better ear until a level was found at which the tone was judged to be equally loud in the two ears (see Fig. 8 for examples). In some patients the fixed tone was presented at a level 40 dB, as well as 20 dB, above the poorer ear threshold.

The ABLB test was usually done at two or three frequencies in the 250- to 4 000-Hz range. In most of the cases where the better ear served as the reference for the loudness matches, the completed laddergrams permitted a ready discernment of the presence or absence of recruitment. Occasionally however the laddergram was not completed because of audiometric limits or because recruitment was clearly evident after only two or three loudness matches. In these instances and in the few instances where recruitment was not immediately evident by inspection of the laddergram, the intensity ranges spanning the equal loudness growth for each ear were compared, and recruitment was presumed to be present when the intensity range for the better ear was at least twice that for the poorer ear. The same procedure in judging presence or absence of recruitment was followed for patients in whom the poorer ear served as the reference. No effort was made to define "partial" versus "complete" recruitment.

In Table 8 (*a–d*) a "Yes" was recorded in the loudness recruitment present column when recruitment was shown at one or more frequencies. Throughout the series, it was noted that when recruitment was present at one testable frequency it was typically present at others. When exceptions to this occurred, they were generally found among the *Retrocochlear Lesion* and *Sudden Loss* patients. In the few instances where only one frequency was tested, whether because of time limitations or because only one frequency was testable, the result for this one freqency was recorded in the table. If recruitment was absent, a "No" was recorded in the table. A blank space in the table indicates one of several possibilities: that no frequencies were testable, that the patient unreliable in his loudness judgments, or that the test simply was not done.

Short increment sensitivity index (SISI)

The SISI test was administered in the manner suggested by Jerger Shedd & Harford (1959) with slight modifications described by Owens (1965). A tone was presented to the affected ear at a level 20 dB above threshold at a given frequency with appropriate masking in the better ear and the patient was asked to signal whenever he heard a superimposed in-

Fletcherian pure-tone average (dB)													
40–43	0				##			##		#			##
36–39		0	0							##	##	####	####
32–35	0	0	0	0						####	c##	### ###	#
28–31	000							#			#	##0	##
24–27				0						##	##	###	####
20–23	0			00					00	#		####	##
16–19		0	0								#	##0	#
12–15					00							0	
8–11													#00
4–7										#			
0–3													#
	0–6	8–14	16–22	24–30	32–38	40–46	48–54	56–62	64–70	72–78	80–86	88–94	96–100

Ménière # disease Other cochlear lesion W-22 speech discrimination score (%)
0 Retrocochlear lesion

Fig 6 W 22 speech discrimination scores in relation to levels of hearing.

55 dB to 80 dB Thus, with Fletcherian averages under 55 dB scores below 20%—especially scores of 0%—would be strongly suspected. Again, judgment should be tempered for elderly patients or patients with extremely sharp slopes in the speech-frequency range

It must be emphasized that the above interpretations are based only on the use of W 22 lists. The differences in interpretation necessitated by the use of other test lists were presented by Carhart in 1965

Regarding the pertinent literature, Schuknecht & Woellner (1955) seem to have been the first to note that a patient with VIIIth nerve lesion may show a reduction in speech discrimination ability that is grossly disproportionate to the degree of pure tone loss. Of two patients whom they reported, one scored only 16% despite normal pure-tone hearing in the speech-frequency range, and the other scored 0% despite only a 42 dB reduction in pure tone sensitivity Goodman (1957) described similar findings in several patients and concluded that a severe speech discrimination loss with normal pure tone thresholds suggests an intracranial lesion. Johnson (1968) reported scores ranging from 0% to 100 with most of them below 30% in 167 patients with retrocochlear lesions. A number of patients scored less than 30% even with normal pure tone thresholds

The Special Tests and their Interpretation

The special test battery comprised the Alternate Binaural Loudness Balance (ABLB) the Short Increment Sensitivity Index (SISI) a tone decay test, and Bekesy tracings.

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For approximately 70% of the patients, the Fowler (1950) technique was followed, in which the tone of fixed intensity was presented to the better ear. At each of several successive 20-dB intensity levels above the threshold in the better ear several pairings of the tone were given. The patient reported verbally for each pairing whether the tone in the poorer ear was louder softer or the same in loudness as the tone in the better ear. The examiner through a bracketing procedure, found the level in the poorer ear at which the tone was judged to be equal in loudness to the tone in the better ear. When this procedure had been completed at several successive 20-dB levels in the better ear a laddergram was drawn which indicated the presence or absence of recruitment. Examples are shown in Fig. 7.

For 30% of the patients, the tone of fixed intensity was presented to the poorer ear (Jerger & Harford, 1960; Jerger 1962). A tone at a level 20 dB above the poorer-ear threshold was paired with tones at varying levels above the threshold for the better ear until a level was found at which the tone was judged to be equally loud in the two ears (see Fig. 8 for examples). In some patients the fixed tone was presented at a level 40 dB, as well as 20 dB above the poorer ear threshold.

The ABLB test was usually done at two or three frequencies in the 250- to 4 000-Hz range. In most of the cases where the better ear served as the reference for the loudness matches, the completed laddergrams permitted a ready discernment of the presence or absence of recruitment. Occasionally however the laddergram was not completed, because of audiometric limits or because recruitment was clearly evident after only two or three loudness matches. In these instances and in the few instances where recruitment was not immediately evident by inspection of the laddergram, the intensity ranges spanning the equal loudness growth for each ear were compared, and recruitment was presumed to be present when the intensity range for the better ear was at least twice that for the poorer ear. The same procedure in judging presence or absence of recruitment was followed for patients in whom the poorer ear served as the reference. No effort was made to define "partial" versus complete recruitment.

In Table 8 (*a–d*) a "Yes" was recorded in the loudness recruitment present column when recruitment was shown at one or more frequencies. Throughout the series, it was noted that when recruitment was present at one testable frequency it was typically present at others. When exceptions to this occurred, they were generally found among the *Retrocochlear Lesion* and *Sudden Loss* patients. In the few instances where only one frequency was tested, whether because of time limitations or because only one frequency was testable, the result for this one freqency was recorded in the table. If recruitment was absent, a "No" was recorded in the table. A blank space in the table indicates one of several possibilities: that no frequencies were testable, that the patient unreliable in his loudness judgments, or that the test simply was not done.

Short increment sensitivity index (SISI)

The SISI test was administered in the manner suggested by Jerger, Shedd & Harford (1959) with slight modifications described by Owens (1965). A tone was presented to the affected ear at a level 20 dB above threshold at a given frequency with appropriate masking in the better ear and the patient was asked to signal whenever he heard a superimposed in-

Fletcherian pure-tone average (dB)													
40–43	0				##			##		#			###
36–39		0	0							##	##	##(0	(,##
32–35	0	0	0	0						####	##	### ###	#
28–31	000							#			#	#(0	##
24–27				0						##	##	###	##
20–23	0			00					00	#		(##	(#
16–19		0	0								#	((0	#0
12–15					00							0	
8–11													# 00
4–7										#			
0–3													0
	0–6	8–14	16–22	24–30	32–38	40–46	48–54	56–62	64–70	72–78	80–86	88–94	96–100

Ménière's disease Other cochlear lesion W 22 speech discrimination score (%)
0 Retrocochlear lesion

Fig 6 W 22 speech discrimination scores in relation to levels of hearing.

55 dB to 80 dB Thus, with Fletcherian averages under 55 dB scores below 20%—especially scores of 0%—would be strongly suspected Again, judgment should be tempered for elderly patients or patients with extremely sharp slopes in the speech frequency range

It must be emphasized that the above interpretations are based only on the use of W 22 lists. The differences in interpretation necessitated by the use of other test lists were presented by Carhart in 1965

Regarding the pertinent literature, Schuknecht & Woellner (1955) seem to have been the first to note that a patient with VIIIth nerve lesion may show a reduction in speech discrimination ability that is grossly disproportionate to the degree of pure tone loss. Of two patients whom they reported, one scored only 16% despite normal pure-tone hearing in the speech-frequency range, and the other scored 0% despite only a 42 dB reduction in pure-tone sensitivity Goodman (1957) described similar findings in several patients and concluded that a severe speech discrimination loss with normal pure-tone thresholds suggests an intracranial lesion Johnson (1968) reported scores ranging from 0% to 100% with most of them below 30% in 167 patients with retrocochlear lesions. A number of patients scored less than 30% even with normal pure tone thresholds

The Special Tests and their Interpretation

The special test battery comprised the Alternate Binaural Loudness Balance (ABLB) the Short Increment Sensitivity Index (SISI) a tone decay test, and Bekesy tracings.

Alternate binaural loudness balance (ABLB)

The ABLB test for loudness recruitment was administered by presenting short-duration (approximately one second) tones of the same frequency alternately from one ear to the other with approximately a one second interval between presentations. The frequencies selected were those showing a normal threshold for one ear (within 25 dB of audiometric zero) and a difference of at least 20 dB between the thresholds for the two ears.

For approximately 70% of the patients, the Fowler (1950) technique was followed, in which the tone of fixed intensity was presented to the better ear. At each of several successive 20-dB intensity levels above the threshold in the better ear several pairings of the tone were given. The patient reported verbally for each pairing whether the tone in the poorer ear was louder softer or the same in loudness as the tone in the better ear. The examiner through a bracketing procedure, found the level in the poorer ear at which the tone was judged to be equal in loudness to the tone in the better ear. When this procedure had been completed at several successive 20-dB levels in the better ear a laddergram was drawn which indicated the presence or absence of recruitment. Examples are shown in Fig. 7

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The ABLB test was usually done at two or three frequencies in the 250- to 4 000-Hz range. In most of the cases where the better ear served as the reference for the loudness matches, the completed laddergrams permitted a ready discernment of the presence or absence of recruitment. Occasionally however the laddergram was *not completed*, because of audiometric limits or because recruitment was clearly evident after only two or three loudness matches. In these instances and in the few instances where recruitment was not immediately evident by inspection of the laddergram, the intensity ranges spanning the equal loudness growth for each ear were compared, and recruitment was presumed to be present when the intensity range for the better ear was at least twice that for the poorer ear. The same procedure in judging presence or absence of recruitment was followed for patients in whom the poorer ear served as the reference. No effort was made to define "partial" versus "complete" recruitment.

In Table 8 (*a–d*) a "Yes" was recorded in the loudness recruitment present column when recruitment was shown at one or more frequencies. Throughout the series, it was noted that when recruitment was present at one testable frequency it was typically present at others. When exceptions to this occurred, they were generally found among the *Retrocochlear Lesion* and *Sudden Loss* patients. In the few instances where only one frequency was tested, whether because of time limitations or because only one frequency was testable, the result for this one freqency was recorded in the table. If recruitment was absent, a "No" was recorded in the table. A blank space in the table indicates one of several possibilities: that no frequencies were testable, that the patient unreliable in his loudness judgments, or that the test simply was not done.

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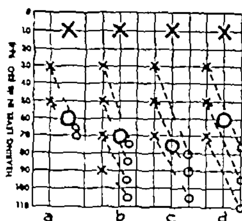


Fig 7 Laddergrams for ABLB when the tone of fixed intensity is presented to the better ear Large X's and O s are thresholds. Loudness recruitment is present in (*a*) (*b*), (*c*) but absent in (*d*).

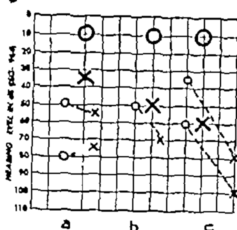


Fig 8 Laddergrams for ABLB when the tone of fixed intensity is presented to the poorer ear Large X's and O s are thresholds. Loudness recruitment present at (*a*) and (*b*), but absent at (*c*).

crement of 1 dB A Yes" was recorded in Table 8 under the SISI column when he scored 60% or higher at one or more *frequencies* Otherwise, a No" was recorded. Although the SISI test was usually given at two or three frequencies circumstances occasionally per mitted the testing of only one frequency As with recruitment, however it had been noted that a high SISI score at one frequency was typically accompanied by high scores at other frequencies, given comparable threshold levels. Therefore if a valid test result was obtained for only one frequency it was recorded in the table

It must be borne in mind that the SISI test often produces low scores with mild threshold losses despite known cochlear involvement (Owens, 1965) In the present survey scores below 60% at frequencies showing threshold levels less than 45 dB were discounted at the same time, scores of 60% or higher at low levels were interpreted as a reflection of cochlear involvement.

In addition the SISI test is sometimes complicated by tone decay in that a patient may lose the carrier tone but continue responding to the 1-dB increments. Hughes (1968) has reported that of a series of 18 patients who demonstrated this behavior three had retrocochlear lesions Therefore, caution must be employed in interpreting the results in these instances.

Whereas the ABLB test was used to explore for the presence of recruitment, the SISI test was used to explore for hyperacuity to small changes in intensity at a given level above threshold A Yes" in either column (Table 8) was interpreted as evidence of cochlear involvement.

Tone decay

The tone decay test employed was one described by Owens in 1964 based on the work of Hood (1955) For a given frequency a tone was presented at a steady intensity 5 dB above the threshold in the poorer ear and the patient was asked to hold up his hand (or press steadily on a signal button) as long as he heard the tone. If the tone faded before a minute had elapsed he was given a 20-sec rest, and the tone was presented at the next higher 5-dB level. This sequence continued until he heard the tone for a minute or until he had listened to the tone at four presentation levels, each 5 dB higher than the preceding one. The time in seconds during which the tone could be heard at each presentation level was noted on the audiogram under the test frequency Examples of various tone decay patterns are shown in Table 7

Table 7 *Examples of tone decay patterns*

Numbers composing tone decay patterns are in seconds

Levels above threshold (dB)	Type I*	Type II				Atypical	Type III					
		A	*B*	*C*	*D*							
5	60	20	9	15	12	3	4	3	2	15	3	36
10		60	32	25	19	10	2	5	12	25	8	40
15			60	40	29	14	5	8	15	20	13	38
20				60	37	19	3	12	10	24	13	42

* No significant tone decay

Type I means no significant tone decay—that is, the patient was able to sustain a tone for 60 sec at 5 dB above threshold. Type II means *that the patient*, after showing decay at the 5-dB level above threshold, was able to sustain the tone for increasingly longer periods of time —on the average of eight seconds or more— at successively *higher levels of presentation* 5 dB apart. Type III means that the patient failed to show any essential increase—less than four seconds, on the average—in the length of time he could sustain the tone with each 5-dB increment "Atypical" means that the tone was heard on the average of four to seven seconds longer with each successive 5-dB increment.

The letter categories in Table 7 are generally not used clinically but they are of aid in describing different kinds of Type II patterns. The patterns are described as follows. "A" the tone decays at 5 dB above threshold, but the 1 min criterion is met at 10 dB "B" the tone decays at 5 and 10 dB above threshold, but is heard noticeably longer at the 10-dB level and for one minute at 15 dB "C" the tone decays at the 5- 10- and 15-dB levels—but is heard noticeably longer with each successive 5-dB increment—and is heard for one minute at 20 dB "D" the tone decays at 5 10 15 and 20 dB above threshold, but is heard for a longer time, on the average of eight seconds or more with each successive 5-dB increment. The "atypical" pattern is synonymous with the "E" pattern of Owens (1964)

White noise masking was introduced to the better ear at a level considered to be *sufficient* even if four or five increments of 5 dB were necessary to complete the test at a given frequency.[1] Shimizu (1969) has reported inducing tone decay (and high SISI scores) in normal and conductively involved ears by applying a moderate amount of contralateral white noise masking. He *attributed the phenomenon* to a neural-interaural interference. Regardless, opposite ear masking was considered to be necessary for the Tone Decay test (as well as for the SISI test) in all instances of unilateral loss in our survey and the results must be interpreted with this understanding. The major question regarding tone decay in cochlear versus retrocochlear involvement—namely whether the tone can be sustained for longer periods *with increased intensity*—was believed not to have been complicated by contralateral masking.

The tone decay test was given at several frequencies, usually those exhibiting the greatest threshold loss. A "Yes" was placed under the appropriate column in Table 8 if at one or more frequencies, the patient showed a Type III pattern. A "No" was inserted if, at all of several frequencies tested, the patient was able to sustain the tone for one minute at any of the four or five intensity levels, or failing this, if he was able to sustain the tone for increasingly longer periods of time—on the aver

[1] *In actual practice, it seems best to limit the tone decay test to four or five intensity increments at each frequency. Further testing sometimes introduces complicating factors such as patient fatigue, confusion, or the necessity for increasing opposite-ear masking.*

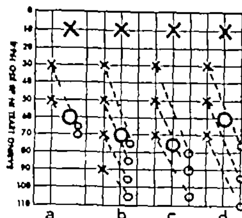


Fig 7 Laddergrams for ABLB when the tone of fixed intensity is presented to the better ear Large X's and O s are thresholds. Loudness recruitment is present in (*a*), (*b*), (*c*), but absent in (*d*).

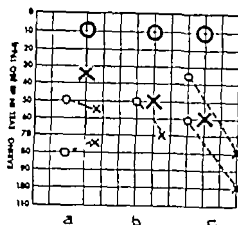


Fig 8 Laddergrams for ABLB when the tone of fixed intensity is presented to the poorer ear Large X's and O s are thresholds. Loudness recruitment present at (*a*) and (*b*), but absent at (*c*).

crement of 1 dB A Yes" was recorded in Table 8 under the SISI column when he scored 60% or higher at one or more frequencies. Otherwise, a No" was recorded. Although the SISI test was usually given at two or three frequencies, circumstances occasionally per mitted the testing of only one frequency As with recruitment, however it had been noted that a high SISI score at one frequency was typically accompanied by high scores at other frequencies, given comparable threshold levels. Therefore, if a valid test result was obtained for only one frequency it was recorded in the table

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In addition, the SISI test is sometimes com plicated by tone decay in that a patient may lose the carrier tone but continue responding to the 1-dB increments. Hughes (1968) has reported that of a series of 18 patients who demonstrated this behavior three had retro-cochlear lesions. Therefore, caution must be employed in interpreting the results in these instances.

Whereas the ABLB test was used to ex plore for the presence of recruitment, the SISI test was used to explore for hyperacuity to small changes in intensity at a given level above threshold. A Yes" in either column (Table 8) was interpreted as evidence of cochlear involvement

Tone decay

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Table 8c. *Patterns of special test results for the* Retrocochlear Lesion *group*

Loudness recruitment present	SISI 60 or greater	Type III tone decay	Significant Békésy shift	No.
No	No	Yes	Yes	15
No	—	Yes	Yes	9
No	—	Yes	—	4
—	No	—	Yes	1
—	N	Yes	—	1
—	No	Yes	Yes	1
No	—	—	Yes	1
—	—	Yes	Yes	5
—	—	Yes	—	4
—	—	—	Yes	3
No	—	—	—	6
No	No	Yes	No	1
No	—	No	No	2
No	—	No	—	1
—	No	No	Yes	1
Yes	—	Yes	Yes	3
Yes	No	Yes	Yes	2
Yes	No	—	Yes	1
Yes	Yes	No	No	9*
Yes	Yes	—	No	4*
—	Yes	No	No	2*
—	—	No	No	1
Yes	Yes	No	—	1
				78

*"Cochlear" special test results.

Stadler E-800 Békésy audiometer provided a tone of gradually changing intensity at the rate of 2.5 dB per second and gradually changing frequency at the rate of one octave per minute. For some patients, sweep-frequency tracings over the range from 250 to 4 000 Hz were obtained, first for an interrupted-tone stimulus (2.5 interruptions per second), then for a continuous-tone stimulus. For other patients, fixed frequency tracings were obtained for interrupted and continuous-tone stimuli at octave-interval frequencies within the same range (250–4 000 Hz). For still other patients, both sweep- and fixed-frequency tracings were obtained. Practice was always given on the non-test ear to ascertain that the task could be accomplished. Subjects were instructed to respond just to the tone, (1) because an occasional patient experienced the "tone perversion" mentioned earlier and (2) because extraneous stimuli seemed to be present at the high-intensity limits of the equipment.

With a substantial hearing loss in the test ear white-noise masking in the better ear was usually set at a level around 100 dB SPL. With near-normal hearing in the test ear the noise level was set at 80 or 90 dB, SPL. The intent here was to guard against overmasking while at the same time ensuring sufficient masking should there be a marked shift in the continuous-tone tracing.

Although both sweep- and fixed-frequency tracings were obtained for a number of patients, time limitations usually necessitated a choice between the two. Problems with the Jerger (1960) classification of the sweep-frequency tracings reported by Owens in 1964 continued in evidence as data accumulated, leading to a preference for the fixed-frequency tracings in the latter part of the survey. For example, at a point in the survey when sweep tracings had been obtained for 76 of the *Ménière's Disease* patients and for 27 of the *Other Cochlear Lesion* patients, a number of patterns were seen in which the continuous-

Table 8d. *Patterns of special test results for the* Sudden Loss *group*

Loudness recruitment present	SISI 60 or greater	Type III tone decay	Significant Békésy shift	No.
Yes	Yes	No	No	10
Yes	Yes	—	No	14
Yes	—	No	No	7
Yes	—	—	No	8
Yes	—	No	—	10
—	Yes	—	No	1
—	Yes	No	No	1
Yes	No	—	No	1
No	Yes	No	No	1
Yes	Yes	Yes	No	1
—	Yes	No	Yes	1
No	—	Yes	—	1
—	No	Yes	Yes	1
No	—	—	Yes	1
No	No	—	Yes	1
No	No	Yes	Yes	4
No	No	No	Yes	1
Yes	Yes	Yes	Yes	1
—	Yes	Yes	Yes	1
Yes	—	Yes	—	2
Yes	—	—	Yes	2
—	No	No	Yes	2
No	Yes	Yes	Yes	1
				73

Table 8*a* *Patterns of special test results for the* Menière s Disease *group*

Loudness recruitment present	SISI 60% or greater	Type III tone decay	Significant Békésy shift	No.
Yes	Yes	No	No	30
Yes	Yes	—	No	14
Yes	—	No	No	25
Yes	—	—	No	12
—	Yes	No	No	3
—	Yes	—	No	8
Yes	—	No	—	20
—	—	No	No	3
—	—	No	—	2
Yes	—	—	Yes	1
Yes	Yes	Yes	Yes	1
Yes	Yes	Yes	No	1
				120

age of eight seconds or more—at each higher intensity level. Where atypical" patterns occurred the results at other frequencies decided the insertion in Table 8

For some patients, a screening test was given The test tone was first presented at a level 10 dB above threshold If the patient failed to sustain the tone for a minute it was presented once again, after a 20-second rest, at a level 10 dB higher If the 60-sec criterion was met at either level a No" was inserted in the table. If the results were questionable, the regular test was given

It should be noted that with any test of tone decay the tonal stimulus may change, for an occasional patient, to a buzz hiss" or other noise instead of disappearing completely This phenomenon, mentioned by Sorensen (1962) and noted by Harbert & Young (1964 *a* 1964 *b*) was termed tone perversion" by Parker Decker & Richards (1968) Its significance is yet to be determined. A few patients in our survey like the one described by Simmons & Dixon (1964) could not discern a tone at all Tone perversion occurred most frequently in the *Sudden Loss* group occasionally in the *Retrocochlear Lesion* group and rarely in the *Menière s Disease* and *Other Cochlear Lesion* groups.

Some patients lost" the test tone, but were able to tell when the switch was turned off typically they reported then that they had still been hearing something. No clear explanation for this phenomenon can be given at present. In a few of these instances, tone perversion seemed to have been operating, while in others the tone was described as having merged with tinnitus. From the patients descriptions, it seemed that the test tone and the tinnitus had become indistinguishable, but that the tinnitus had somehow been altered in the process. Consequently when the tone was turned off they became aware of another change in the tinnitus. For most patients in the *Menière s Disease* and *Other Cochlear Lesion* groups, Type II patterns typically emerged despite tinnitus that is, at successively higher presentation levels, the test tone took longer to blend with the tinnitus. In a few of the cochlear lesion patients however an apparently loud tinnitus interfered to produce Type III tone decay patterns at certain frequencies. Such results were of course not valid The data reported in the present survey are based on the premise that the patient, as instructed, ceased responding when the test tone disappeared.

Békésy tracings

Békésy tracings were obtained as described by Jerger (1960 *a*) and Owens (1964) A Grason–

Table 8*b* *Patterns of special test results for the* Other Cochlear Lesion *group*

Loudness recruitment present	SISI 60 or greater	Type III tone decay	Significant Békésy shift	No.
Yes	Yes	No	No	15
Yes	Yes	—	No	18
Yes	—	No	No	7
Yes	—	—	No	4
Yes	—	No	—	6
—	Yes	—	No	5
Yes	Yes	Yes	No	
Yes	—	Yes	No	1
Yes	No	No	No	1
Yes	No	—	No	1
Yes	Yes	Yes	Yes	
				6[illegible]

from the midpoint of the (*I*) tracing width (19 9 dB for the *Menière's Disease group* 19.5 dB for the *Other Cochlear Lesion group*)—the pattern was considered *uninterpretable*. When audiometer limits were reached after a shift *of more* than 20 dB the tracing was considered to be significant because the (*C*) tracing with a cochlear loss would then be expected to stabilize only about five times in 100 were sufficient room available. Occasionally the (*C*) tracing for the *Retrocochlear Lesion* group was uninterpretable at 250 Hz, but interpretable at higher frequencies where the audiometer limits were wider In Table 8, then, a "Yes" was recorded under Significant Békésy Shift" when (*C*) reached audiometer limits after a shift of 20 dB or more.

The problem of masking with sweep-frequency tracings deserves special mention. On the one hand, the masking may be of sufficient intensity for the (*I*) tracing of the test ear but not for the (*C*) tracing when a downward shift occurs (since the intensity of the test tone then increases until it overcomes the opposite-ear masking) a shadow tracing—a pseudo Type IV—may thus be produced. On the other hand, a high level of opposite-ear masking may through some as yet unexplained mechanisms, selectively shift (*C*) without concomitantly shifting (*I*)

Apropos is a recent study by Blegvad (1968) who used the Békésy audiometer (Grason Stadler *E-800*) used in our study and followed the same general test procedure except that he employed *inserts* instead *of* standard earphones for white-noise masking. He observed the Békésy tracings for the affected ears of 31 patients with unilateral sensorineural loss under conditions of no masking and of 80-dB masking, being careful to rule out shadow curves in the unmasked condition and overmasking in the masked condition. He found noticeable differences in the relation between (*I*) and (*C*) under the two conditions. For example, *the* masking often changed Type I tracings to Type II, Type II to Type IV and, in three patients, Type I to Type IV Also, the masking frequently changed the relation between (*I*) and (*C*) with respect to the amplitude of the tracings. No explanation of these changes was offered. Although Blegvad stated in effect that these findings fail to detract from the diagnostic significance of Békésy audiometry his findings are nevertheless unsettling to the conscientious audiologist. The Békésy *results of the* present survey must be interpreted on the basis that masking was employed *for all subjects*. The extent of pure-tone sensorineural loss in *most of the* affected ears *made it unlikely that over*masking occurred, even with the white noise at a *maximum tolerable* level (usually 100 to 110 dB, SPL) in the non-test ear The possibility that overmasking occurred with near normal pure-tone levels in the affected ear cannot be ruled out entirely

Two other troubling aspects of masking with sweep-frequency tracings are: (*a*) the most appropriate level of masking changes as the frequency range is traversed, and (*b*) a high level of masking for the 4 or 5 min required to complete a sweep may be discomforting and possibly harmful to the patient.

The use of *fixed-frequency tracings* tended to reduce problems of interpretation. In these tracings for patients with cochlear lesions, either (*C*) and (*I*) overlapped (Jerger Type I) or (*C*) shifted downward slightly with narrowing of width and stabilization in a position parallel to (*I*) (Jerger Type II). When fixed-frequency Békésy tracings had been obtained for 55 *Menière's Disease* patients and 43 *Other Cochlear Lesion* patients, the shifts were measured from the midpoint of each (*I*) tracing to the midpoint *of the* corresponding (*C*) tracing. The means of the shifts and their *S.D.s* appear in Table 10 In calculating the *means*, 0-dB shifts (Jerger Type *I*) were not counted. For the frequencies 500, 1 000 2 000 and 4 000 Hz, the means for the *Menière's Disease* group were 7.3 7.5 8.8, and 8 8 dB S.D.s were 4.7 2.8, 3.3, and 4 0 dB. The means for *the Other Cochlear Lesion* group at the same *frequencies* were 7 4 8.6, 9.3 and 10.4 dB S.D.s were 3.6, 3 4 4.5 and 5 6 dB. The

t.

tone tracing (C) shifted downward from the interrupted-tone tracing (I) at or below 250 Hz and remained below the (I) tracing over the entire frequency range to 4 000 Hz. As (C) *shifted, it narrowed noticeably in* width compared to (I) These patterns were the Jerger Type IV that have been associated with retrocochlear involvement. They appeared in 20 of the 76 tracings for *Menière s Disease* patients, and in eight of the 27 tracings for the *Other Cochlear Lesion* patients, but in none of 50 tracings obtained for the *Retrocochlear Lesion* patients. Such patterns are thus clearly consistent with *cochlear* involvement. Hughes Winegar & Crabtree (1967) have also concluded that the Jerger Type IV patterns as well as the Jerger Type II patterns, generally represent cochlear impairment.

In addition, other patterns only briefly mentioned in the Jerger (1960) classification system appeared in eight of the same 76 patients in the *Menière s Disease* group and in one of the same 27 patients in the *Other Cochlear Lesion* group In these patterns, the (*C*) tracing shifted downward from the (*I*) tracing in the low tone range (beginning around 250 Hz) and rejoined the (*I*) tracing in the middle or high-tone range A narrowing of tracing width typically accompanied the (C) shift. Generally these patterns were associated with pure-tone configurations showing noticeably greater low tone than high tone impairment. No pattern of this kind occurred in the 50 sweep tracings obtained for the *Retrocochlear Lesion* group

Accordingly the definition of Owens (1964) was followed regarding Type II (cochlear) sweep tracings. the (*C*) training may drop below (*I*) at any point in the frequency range (250–4 000 Hz) typically with concomitant narrowing It then characteristically remains essentially parallel to (*I*) but occasionally may rejoin (*I*)" The interpretation of the amount of shift in such tracings remains a problem, however Hughes et al (1967) attached a retrocochlear interpretation to tracings in which (*C*) parallels (*I*) at a distance greater than 20 dB Adherence to this limitation would misclassify as retrocochlear" two of our *Other Cochlear Lesion* and one of our *Sudden Loss* patients. The latter experienced a sudden loss in his operated ear several months after stapedectomy Other special test findings for all three patients were cochlear" and the sweep Békésy (*C*) tracings which were narrower in width than (*I*) ran parallel to (*I*) at a distance between 20 and 25 dB below Accordingly 25 dB might be a more practical limit beyond which retrocochlear lesion would be suspected. This corresponds to approximately three standard deviations below the mean shift of (*C*) measured for a group of cochlear lesion patients—see next section. (It is assumed that we are not discussing a transient (*C*) shift, perhaps greater than 30 dB that may occur at one point after which (*C*) approaches (*I*) These seemingly erratic shifts with narrowing of width occurred in two of our *Other Cochlear Lesion* patients.) On the other hand, we would suspect retrocochlear involvement whenever narrowing fails to occur with a shift of (*C*) since in only two or three instances did the (*C*) tracings of the *Menière s Disease* + *Other Cochlear Lesion* patients fail to narrow perceptibly

Another problem with the sweep-frequency tracings was that when hearing impairment was severe (*C*) often reached audiometer limits after only a slight shift. It would have been inaccurate to label such a tracing a Jerger Type III (retrocochlear) pattern when the tracing might have stabilized to a Type II (cochlear) pattern given more time and space. For the 76 *Menière s Disease* patients and the 27 *Other Cochlear Lesion* patients, the amount of shift was measured at *the point of greatest* shift. Measurements were from the midpoint of (*I*) to the midpoint of (*C*) For the *Menière s Disease* group, the mean of these shifts was 10 5 dB and the standard deviation (S.D) was 4 7 dB for the *Other Cochlear Lesion* group the mean was 10 7 dB and the S D 4 4 dB (Table 9)

Accordingly when (*C*) reached audiometer limits after shifting less than two S.D s from the mean shift—that is less than about 20 dB

fect, that the fixed-frequency tracings were chosen in all cases of disagreement.

Regarding the relative widths of the (*I*) and (*C*) tracings, (*C*) almost invariably narrowed in the process of shifting the small amounts that indicated cochlear involvement. This narrowing occurred in both the sweep- and fixed-frequency tracings. In the sweep-frequency shifts significant for retrocochlear involvement, (*C*) usually progressed steadily downward to audiometer limits, so that narrowing of tracing width, although it occurred sometimes, could not be measured in the manner employed for the tracings that stabilized. For 37 patients in the *Retrocochlear Lesion* group whose fixed-frequency tracings indicated retrocochlear involvement, (*C*) shifted steadily to audiometer limits (Jerger Type III) at all frequencies tested (20 patients). Type III shifts occurred at some frequencies while at other frequencies (*C*) shifted and then stabilized (eight patients) and Type III shifts failed to occur but (*C*) shifted an amount significant for retrocochlear involvement and then stabilized (nine patients). Where (*C*) shifted significantly and then stabilized, narrowing of tracing width occurred in about one-half of the instances. In these last 17 of the 37 patients, "cochlear" tracings (Jerger Type II, i.e., insignificant shifts) were sparsely scattered throughout the records.

Fixed-frequency patterns in which (*C*) stabilizes after shifting an amount that would indicate retrocochlear involvement can reasonably be labeled Type IV whether or not narrowing occurs. The use of such labeling would recognize Hopkinson's (1966) plea that some sort of standardization based on Jerger's original descriptions be maintained, if classifications must be used. When (*C*) *fails to narrow* in the process of stabilizing, retrocochlear involvement should be suspected even though the amount of shift does not meet the significance criterion. Such tracings might be labeled "atypical."

Results of Special Tests

The complete results for the special tests are shown in Table 8 (a–d). As mentioned previously, a number of patients did not receive all tests. Occasionally testing was discontinued owing to illness or fatigue of the patient. In other instances, limitations of time prevented the completion of all the tests and the patient failed to return as requested. In still other instances, the pure-tone levels precluded valid ABLB and SISI tests and, finally, some patients could not perform reliably on all tests. Since these circumstances are no doubt common to most clinics, with the consequent necessity of sometimes basing impressions on incomplete test findings, it seemed appropriate to include all patients who had given reliable responses for at least one of the special tests.

A helpful factor in dealing with an incomplete test battery was the close agreement (see Table 8) between the ABLB and SISI tests and also between the Tone Decay and Békésy tests. This agreement had been noted previously for the ABLB and SISI tests by Yantis & Decker (1964) and Owens (1965) and for the Tone Decay and the Békésy tests by Harbert & Young (1964 *b*) and Owens (1965).

Both the ABLB and the SISI tests were given to 151 patients, some from each group. The results agreed in 144 instances—that is, both were designated "Yes" or both were designated "No" in Table 8. In most of the disagreements, one or both of the tests gave indefinite results, e.g., questionable recruitment, or SISI scores slightly below 60%. Both the Tone Decay and the Békésy tests were given to 174 patients, some from each group, and the results agreed in 163 instances. In seven of the 11 patients showing disagreement, the Tone

Table 9 *Békésy shifts with cochlear lesions (sweep-frequency tracings)*

Group	No of tracings	Mean shift (dB)	S.D (dB)
Menière s Disease	76	10.5	4 7
Other Cochlear Lesion	27	10.7	4 4

* The shift for each tracing was measured at the point of greatest shift and from midpoint of (*I*) to midpoint of (*C*).

Ns for the tracings differed among the frequencies because the same frequencies were not tested for all patients. At 250 Hz, too few patients were tested to justify the computation of means and standard deviations.

In the *Menière s Disease* group one patient showed shifts of 50 dB and 60 dB at 2 000 and 4 000 Hz. In the *Other Cochlear Lesion* group one patient showed shifts of 33 dB and 28 dB at 250 and 500 Hz. These two patients were judged to be atypical and were excluded from Table 10

The mean shifts and the S D.s of the *Other Cochlear Lesion* groups were employed in the interpretation of fixed frequency tracings.[1] For a given frequency a shift was considered significant of retrocochlear involvement when it fell more than three S.D s below the mean shift. In round numbers, shifts of three S D.s from the mean were synonymous with shifts of 18 19 23 and 27 dB measured from the mid points of the (*I*) tracings at 500 1 000 2 000 and 4 000 Hz A Yes was recorded in Table 8 when a significant shift occurred at one or more frequencies. For patients showing no shifts, or shifts less than 18 19 23 or 27 dB before stabilization a No" was recorded in Table 8 The interpretation differed slightly when (*C*) reached audiometer limits. If (*C*) shifted more than two S D s from the mean for any test frequency before reaching audiometer in limits the shift was considered significant and a Yes" was inserted in Table 8. If (*C*) shifted less than two S D.s before reaching audiometer limits the shift was considered to be uninterpretable for the test frequency In round numbers shifts of two S D.s for 500 1 000 2 000 and 4 000 Hz were 15 15 18, and 22 dB from the midpoints of the (*I*) tracings.

Inevitably the question arose whether to use the fixed or sweep-frequency tracings in localization of lesion when both had been done When both tests were interpretable results usually pointed to the same decision regarding a "Yes" or No" in Table 8 Both kinds of tracings were obtained for 37 *Menière s Disease* patients with the results agreeing in 36 for 16 patients in the *Other Cochlear Lesion* group with the results agreeing in 14 for 36 patients in the *Retrocochlear Lesion* group with the resulting agreeing in all but four and for 19 *Sudden Loss* patients with the results agreeing in all but one. In all instances when the results disagreed the fixed tracings indicated a Yes" and the sweep tracings a No in Table 8

When no interpretable results were obtained, a blank was left in the table When disagreement occurred between interpretable sweep- and fixed frequency tracings, we selected the Yes" result for the table This meant, in ef

Table 10 *Békésy shifts with cochlear lesions (fixed-frequency tracings)*[a]

Frequencies (Hz)	No. of tracings	Mean shift (dB)	S.D (dB)
Menière s Disease			
500	28	7 3	4.7
1 000	46	7 5	2.8
2 000	52	8.8	3.3
4 000	45	8.8	4.0
Other Cochlear Lesion			
500	23	7 4	3 6
1 000	29	8.6	3.4
2 000	36	9.3	4.5
4 000	38	10.4	5 6

[a] Measured from midpoint of (*I*) to midpoint of (*C*).

[1] Since these means and S.D s were consistently greater than those for the *Menière s D sease* group, it was reasoned that est mates of sign ficant shifts based on data for the *Other Cochlear Lesion* group would apply to the *Menière s Disease* group as well.

fect, that the fixed-frequency tracings were chosen in all cases of disagreement.

Regarding the relative widths of the (*I*) and (*C*) tracings, (*C*) almost invariably narrowed in the process of shifting the small amounts that indicated cochlear involvement. This narrowing occurred in both the sweep- and fixed-frequency tracings. In the sweep-frequency shifts significant for retrocochlear involvement, (*C*) usually progressed steadily downward to audiometer limits, so that narrowing of tracing width, although it occurred sometimes, could not be measured in the manner employed for the tracings that stabilized. For 37 patients in the *Retrocochlear Lesion* group whose fixed-frequency tracings indicated retrocochlear involvement: (*C*) shifted steadily to audiometer limits (Jerger Type III) at all frequencies tested (20 patients). Type III shifts occurred at some frequencies while at other frequencies (*C*) shifted and then stabilized (eight patients) and Type III shifts failed to occur but (*C*) shifted an amount significant for retrocochlear involvement and then stabilized (nine patients). Where (*C*) shifted significantly and then stabilized, narrowing of tracing width occurred in about one-half of the instances. In these last 17 of the 37 patients, "cochlear" tracings (Jerger Type II, i.e., insignificant shifts) were sparsely scattered throughout the records.

Fixed-frequency patterns in which (*C*) stabilizes after shifting an amount that would indicate retrocochlear involvement can reasonably be labeled Type IV whether or not narrowing occurs. The use of such labeling would recognize Hopkinson's (1966) plea that some sort of standardization based on Jerger's original descriptions be maintained, if classifications must be used. When (*C*) fails to narrow in the process of stabilizing, retrocochlear involvement should be suspected even though the amount of shift does not meet the significance criterion. Such tracings might be labeled "atypical".

Results of Special Tests

The complete results for the special tests are shown in Table 8 (*a–d*). As mentioned previously a number of patients did not receive all tests. Occasionally testing was discontinued owing to illness or fatigue of the patient; in other instances, limitations of time prevented the completion of all the tests and the patient failed to return as requested; in still other instances, the pure-tone levels precluded valid ABLB and SISI tests; and, finally, some patients could not perform reliably on all tests. Since these circumstances are no doubt common to most clinics, with the consequent necessity of sometimes basing impressions on incomplete test findings, it seemed appropriate to include all patients who had given reliable responses for at least one of the special tests.

A helpful factor in dealing with an incomplete test battery was the close agreement (see Table 8) between the ABLB and SISI tests and also between the Tone Decay and Békésy tests. This agreement had been noted previously for the ABLB and SISI tests by Yantis & Decker (1964) and Owens (1965) and for the Tone Decay and the Békésy tests by Harbert & Young (1964 *b*) and Owens (1965).

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Table 11 *Special test results for all groups*

Loudness recruitment present and/or SISI greater than 60 %	Type III tone decay and/or significant Békésy shift	No.
(a) Menière's Disease		
Yes	No	112
—	No	5
Yes		1
Yes	Yes	2
		120
(b) Other Cochlear Lesion		
Yes	No	55
Yes	×	3
×	No	2
Yes	Yes	2
		62
(c) Retrocochlear Lesion		
No	Yes	32
Yes	No	16[a]
Yes	Yes	3
No	No	3
×	Yes	3
—	Yes	12
No	—	6
—	No	1
No		2
		78
(d) Sudden Loss		
Yes	No	51
Yes	Yes	6
No	Yes	8
Yes	×	2
×	No	[illegible]
No	×	3
	Yes	1
		73

[a] "Cochlear" special test results.

Decay test indicated retrocochlear involvement, while the Békésy test indicated cochlear involvement in two of these seven, the disagreement occurred only at 4 000 Hz. In the remaining four of the 11 patients, the reverse was shown, the Tone Decay test indicating cochlear involvement, and the Békésy retrocochlear involvement.

Accordingly with the two tests of each pair largely in agreement regarding cochlear versus retrocochlear lesion, the results shown in Table 8 were distilled into those of Table 11 for clearer exposition In that table, discrepancies between the ABLB and SISI and between the Tone Decay and Békésy tests are indicated by an "X"

Of 120 *Menière's Disease* patients, "Yes-No" results occurred for 112, "-No" for five, "Yes-X" for one, and "Yes-Yes" for two. Of the 62 *Other Cochlear Lesion* patients "Yes-No" results occurred in 55 "Yes-Yes" in two, "X-No" in two, and "Yes-X" in three. The combined results for all 182 patients with presumed cochlear involvement were "Yes-No" in 167 "Yes-Yes" in four "X-No" in two, "Yes-X" in four and "-No" in five. "Yes-No" results are thus characteristic of cochlear involvement

Special tests were given to 78 patients in the *Retrocochlear Lesion* group (Table 11 *c*) This group included the five previously mentioned patients who were omitted from the pure-tone pattern section because their pure tone results could not be classified. Excluded from this group because they had had no special tests were three patients who had been included in the pure-tone section "No-Yes" results predominated, occurring in 32 of the 78 patients. Note that 13 patients showed neither ABLB nor SISI test results. The special test results for these 13 were "-Yes" for 12 and "-No" for one. Seven of the 13 had normal or near normal hearing in the affected ear rendering standard ABLB and SISI testing invalid. In the remaining six of the 13 neither the ABLB nor the SISI tests could be done for the various clinical reasons mentioned earlier Six other patients, "No-" in Table 11 *c* had neither the Békésy nor the Tone Decay test. Other results for the *Retrocochlear Lesion* group were "No-No" in three "Yes-Yes" in three, "X-Yes" in three, "No-X" in two and "Yes-No" in 16

According to Table 11 it seemed reasonable to class pairings of "Yes-X" "X-No" "-No" and "Yes-No" as "cochlear" and to class pairings of "X-Yes" "-Yes" "No-" "No-X" and "No-Yes" as "retrocochlear" Thus where an X occurred for one pair of tests, the findings were classed according to the results for the other pair "Yes-Yes" and "No-No" pairings were classed as "mixed cochlear-retrocochlear" In summary then two patients in the *Menière's*

Disease group and two patients in the *Other Cochlear Lesion* group, all "Yes-Yes" showed mixed cochlear-retrocochlear findings—that is, 2.2% of the 182 patients in the combined cochlear lesion groups. All the rest (97.8%) showed cochlear signs. On the other hand, 17 of the 78 patients (22%) in the *Retrocochlear Lesion* group, one with a "X-No" pairing and 16 with "Yes-No" pairings, showed cochlear findings. Fifty-five patients (70%) in the *Retrocochlear Lesion* group showed retrocochlear signs, and the remaining six patients (8%) showed mixed cochlear-retrocochlear findings. The fact that mixed cochlear-retrocochlear findings were relatively infrequent in the cochlear lesion patients compared to the retrocochlear lesion patients suggests the advisability of stressing the likelihood of retrocochlear involvement whenever mixed results are seen.

In the *Sudden Loss group*, 55 patients (75%) showed cochlear results (51 "Yes-No," two "X-No" and two "Yes-X"), 12 patients (17%) showed retrocochlear results (eight "No-Yes" three "No-X" and one "X-Yes") and six (8%) showed mixed cochlear-retrocochlear results (all "Yes-Yes").

Of major interest are the 17 patients in the Retrocochlear Lesion group whose special test results were completely cochlear. These patients are marked by asterisks in Tables 8-c and 11-c. In the one patient (with a brain-stem glioma) who had only a Békésy test, minimal hearing loss precluded standard ABLB and SISI testing. Although both sweep and fixed Békésy tracings were Type I, retrocochlear involvement was correctly predicted for this patient when the discrimination score for the poorer ear on a W-22 list distorted by noise was dramatically reduced by 74 points in contrast to a reduction of only 18 points for the opposite ear (see discussion of Goetzinger & Angell, 1965). All results for the other 16 patients indicated cochlear involvement. Recruitment was present at all testable frequencies in all but two patients, these showing complete recruitment at one frequency and no recruitment at others. (The recruitment findings for these two patients, in retrospect, should have been interpreted as suggestive of retrocochlear involvement—see Discussion.) SISI scores were typically 100%. Tone Decay was Type I or II and Békésy tracings, whether fixed or sweep, showed no significant shifts of the (C) tracing. The slight (C) shifts that did occur were accompanied by a noticeable narrowing of width. Sweep Békésy tracings were obtained for nine of these 16 patients, fixed tracings for six, and both kinds for one. In the nine sweep tracings, shifts of (C) occurred after 500 Hz, if at all. Thus the tracings fitted Jerger's (1960) Type II definition. The discrimination scores all seemed consistent with the extent and pattern of pure tone loss. It is noteworthy that *all* special tests were misleading.

A variety of retrocochlear lesions were involved in these 17 patients: sheath tumors (10), arachnoid cyst (4), meningioma (1), brain-stem glioma (1) and granulomatous meningitis (1).

Other patients of special interest in the *Retrocochlear Lesion* group are some of those for whom neither the ABLB nor the SISI tests could be done in the ordinary sense. Groupings for discussion are as follows: (1) those with unilateral retrocochlear involvement whose pure-tone patterns could not be classified according to our system (four patients) and (2) those who demonstrated bilateral Type *III* tone decay or significant Békésy (C) shifts with either unilateral pure-tone loss or normal hearing for pure tones bilaterally (three patients).

(1) Two of the patients with *unclassifiable* pure-tone audiograms had normal and equal pure-tone hearing bilaterally. One of these had multiple sclerosis and the other an epidermoid cyst of the pons involving cranial nerves V and VII–XI on the left. For both patients, Békésy fixed-frequency tracings supported normal hearing in one ear but indicated retrocochlear involvement in the other. It should be noted that the *sweep* Békésy (*I*) and (*C*) tracings *for the affected* ears overlapped, showing nothing of significance. Surprisingly, 2 days after pneumoencephalographic air studies, the fixed-frequency Békésy (*I*) and (*C*) tracings over

lapped completely for the affected ear in the patient with the cyst since this patient was highly reliable it was thought that the air studies had possibly displaced the cyst.

The third patient of this group, with a meningioma in the tentorial notch extending to involve the VIIIth nerve on the right side, had a mild bilateral high-tone loss showing no consistent difference between the ears. For the right ear Type III tone decay occurred at several frequencies sweep- and fixed frequency Békésy tracings showed significant shifts to audiometer limits, and a W 22 score of 20% occurred with a pure-tone speech-frequency average of 18 dB For the left ear neither tone decay nor Békésy (*C*) shifts occurred and the speech discrimination score was 98%

The fourth patient of this group had a mild, gradually sloping pure tone loss in the right ear associated with a brain-stem glioma. Pure tone thresholds for the left ear when compared with those for the right ear were the same (15 dB) at 250 and 500 Hz, 15 to 20 dB better at 1 000 and 2 000 Hz, and 35 to 40 dB poorer at 4 000 and 8 000 Hz, thus producing a reversal of configurations The history revealed exposure to gunfire and bomb blasts Special test findings for the left ear were consistent with normal hearing in the low and middle frequencies, and cochlear involvement at 4 000 Hz. For the right ear Tone Decay was Type II at 500 Hz and Type III from 1 000 through 4 000 Hz. Although the sweep (*I*) and (*C*) Békésy tracings interwove (Type I) fixed Békésy tracings showed (*C*) shifting slightly at 500 Hz, inconclusively at 1 000 Hz, and significantly at 2 000 and 4 000 Hz.

(2) Of the three patients in this second group one with brain-stem glioma demonstrated a mild gradually sloping, pure tone loss in the left ear and pure-tone hearing just within normal in the right. W 22 scores were 0% in the left ear 68% in the right Both ears showed Type III Tone Decay and significant Békésy shifts at several frequencies. The second patient, with multiple sclerosis, showed tone decay at all frequencies in both ears in the 250–4 000 Hz range although she had normal and equal pure tone hearing bilaterally In each ear Tone Decay was Type III for one frequency and either Type II or atypical for the rest. Although the sweep Békésy (*I*) and (*C*) tracings overlapped completely the fixed-frequency (*C*) tracings for both ears shifted slightly at all frequencies and significantly at 2 000 Hz. Little or no narrowing occurred. W 22 scores were 96% bilaterally The third patient, with a left cerebellopontine angle epidermoid cyst extending through the foramen magnum to the right, showed for the left ear a severe pure tone loss, 0% speech discrimination Type III tone decay and significant Békésy shifts. Although the right ear showed normal pure tone hearing and 96% speech discrimination, Type III tone decay occurred in that ear at 2 000 and 4 000 Hz.

In connection with this second group, a patient described by Reger (1962) should be mentioned. This patient demonstrated marked Békésy shifts bilaterally although bilateral pure tone hearing was within the normal range. A massive pinealoma was found at operation, and the Békésy tracings reverted to normal after its removal.

Obviously the findings for these two small groups of patients point up the importance of bilateral testing for at least tone decay and Békésy shifts, despite normal pure tone hearing, when patients are referred on suspicion of retrocochlear involvement Furthermore, they clearly show that the Békésy fixed-frequency tracings are more sensitive to retrocochlear involvement than the sweep-frequency tracings (see also Katinsky & Toglia, 1968)

During the period of this survey many patients referred by otolaryngological neurological and neurosurgical services for the ruling out of retrocochlear involvement of the auditory pathways were found to have normal hearing for pure tones and speech with no evidence of tone decay or significant Békésy shifts. Some of these patients showed Type II Békésy tracings, as might have been expected from the report of Price et al. (1965) No particular

meaning was attached to these minimal shifts with respect to the question of cochlear versus retrocochlear involvement. A number of patients were also tested for a difference between the two ears with respect to loudness growth and performance on a difficult test of speech discrimination employing noise or low-pass filtering (see Jerger 1960 Goetzinger & Angell, 1965 and Hodgson, 1967). Results of these tests were negative for retrocochlear involvement. Common symptoms of these patients were vestibular disturbances, headaches, and facial tics. Complete medical and neurological work-ups were negative with respect to neoplasm, and in most instances no final diagnosis was made, or the problem was described as "emotionally based". On the other hand, an occasional patient in this group was found to have a tumor that seemingly should have involved the auditory pathways, but such involvement was not reflected in the Tone Decay or Békésy tests. (ABLB testing, when performed showed nothing of significance). Special tests of greater power appear to be needed here (See, for example Sanchez Longo et al. 1957 Bocca, 1958 Eichel et al 1966 Jerger 1960 Katz et al., 1963).

With respect to the *Sudden Loss* patients, the question arises whether a retrocochlear lesion in the form of a neoplasm might have been present in the 18 patients whose special test results suggested either retrocochlear or mixed cochlear-retrocochlear involvement. Twelve of the 18 had had roentgenograms of the internal auditory meati, all of which had been clearly within normal limits. A marked improvement in hearing occurred subsequently in one of the six who had had no roentgenograms, and findings on caloric tests and neurological examination were normal in one other patient. Of the remaining four who had not had roentgenograms, three had had a severe hearing loss for several years with no other symptoms. In this connection, it should be noted that in all 18 patients, the hearing loss was severe at onset.

Thus, at present we believe it unlikely that a retrocochlear lesion in the form of neoplasm would be associated with sudden hearing loss as defined in this survey even though the special test results might sometimes be the same as those indicating a neoplasm (see Jerger et al 1961).

As yet there is no real agreement on the etiology or localization of lesion in sudden loss. Until there is, the audiologist must qualify to the best of his ability the "retrocochlear" special test results that are sometimes encountered. At present he can only suggest that such findings do appear occasionally in patients with a history of sudden loss, and that, regardless of the special test findings, neoplasm has been a rare occurrence in several reported series. Thus the history of onset assumes major importance.

Relation between ABLB/SISI and Tone Decay/Békésy

The close agreement between the ABLB and SISI and between the Tone Decay and Békésy tests with respect to site of lesion has already been discussed. In this section we will consider the agreement between the two pairs of tests.

For the combined *Ménière's Disease + Other Cochlear Lesion* group, the ABLB SISI results and the Tone Decay Békésy results showed good agreement regarding site of lesion. As may be seen in Table 11 discrepancies ("Yes-Yes") occurred in only four instances (2%). In two of the four ABLB/SISI and Tone Decay/Békésy were done at the same frequencies. In the other two ABLB/SISI were done at certain frequencies and Tone Decay/Békésy at other frequencies. The findings for these two patients were unusual in that, as mentioned previously the indications for site

lapped completely for the affected ear in the patient with the cyst since this patient was highly reliable it was thought that the air studies had possibly displaced the cyst.

The third patient of this group with a meningioma in the tentorial notch extending to involve the VIIIth nerve on the right side, had a mild bilateral high-tone loss showing no consistent difference between the ears For the right ear Type III tone decay occurred at several frequencies, sweep- and fixed frequency Békésy tracings showed significant shifts to audiometer limits, and a W 22 score of 20% occurred with a pure tone speech-frequency average of 18 dB For the left ear neither tone decay nor Békésy (C) shifts occurred and the speech discrimination score was 98%

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In connection with this second group, a patient described by Reger (1962) should be mentioned. This patient demonstrated marked Békésy shifts bilaterally although bilateral pure tone hearing was within the normal range. A massive pinealoma was found at operation, and the Békésy tracings reverted to normal after its removal

Obviously the findings for these two small groups of patients point up the importance of bilateral testing for at least tone decay and Békésy shifts despite normal pure-tone hearing, when patients are referred on suspicion of retrocochlear involvement Furthermore, they clearly show that the Békésy fixed frequency tracings are more sensitive to retrocochlear involvement than the sweep-frequency tracings (see also Katinsky & Toglia, 1968)

During the period of this survey many patients referred by otolaryngological, neurological, and neurosurgical services for the ruling out of retrocochlear involvement of the auditory pathways were found to have normal hearing for pure tones and speech, with no evidence of tone decay or significant Békésy shifts Some of these patients showed Type II Békésy tracings as might have been expected from the report of Price et al. (1965) No particular

between the ears or by unreliability of test results, especially if tone perversion was involved.

On their return visits, patients with subsequently verified retrocochlear lesions frequently showed a worsening of pure-tone thresholds. Of ten *Retrocochlear Lesion* patients who returned for retests before operation, the following downward shifts in pure-tone thresholds were noted: the shifts were fairly even across the frequency range (four patients) the shifts were confined primarily to the high frequencies (two patients) the shifts were confined to the low (250–500 Hz) frequencies (two patients) and the shifts occurred at selective frequencies (one patient). No shifts occurred in one patient. Thus, although downward shifts in hearing, shown on repeated examination, are strongly indicative of retrocochlear involvement (see Bucy & Ismat, 1961) little reliance can be placed upon the *pattern* of shift. The pattern of the shift further loses significance when one notes (see Goodman, 1965) that successive changes in pure-tone configurations for Menière's disease (cochlear) patients reveal no characteristic pattern that is, a given pattern of shift between two successive tests might occur with either cochlear or retrocochlear lesions.

Irrespective of the pattern of shift, though, it would seem unwise to consider a progressively downward shift in hearing, per se to be an invariable sign of retrocochlear lesion in the form of neoplasm. For example, factors such as labyrinthine otosclerosis and systemic, inflammatory or viral disorders affecting the cochlea would have to be considered, and, as Davis (1962) points out, toxic or metabolic factors may interfere with VIIIth-nerve function.

Discrimination scores for the above ten retested *Retrocochlear Lesion* patients decreased variably In four of the ten, however scores decreased by 70 points or more, greatly out of proportion to the pure-tone shift, thereby providing strong evidence for retrocochlear involvement.

With respect to the special tests, a few *Retrocochlear Lesion* patients showed predominantly cochlear signs on one visit and predominantly retrocochlear signs on a visit several months later Conversely one patient with a sheath tumor had definite retrocochlear signs on one visit and definite cochlear signs on the next.

One of the two Menière's Disease patients, who showed "Yes–Yes findings in Table 11 revealed completely cochlear signs on the same tests five months later accompanied by pure tone improvement on the order of 10 to 15 dB. Finally in two of the *Sudden Loss* patients, special test results changed from retrocochlear to cochlear in conjunction with noticeable improvement in pure-tone thresholds. In this connection, Harbert & Young (1964 *a*) have reported on two patients with sudden loss, one with cochlear and the other with retrocochlear special test findings, both of whom experienced a complete recovery of normal hearing.

In any event, the important point is that questionable findings regarding site of lesion can often be resolved when hearing is retested after a few weeks or months. Changes may occur in pure-tone thresholds, in discrimination scores, or in the patterning of special test results.

Constellations of Test Results

Doubtless, information regarding site of lesion accrues when the special test findings are viewed together with pure-tone patterns and speech discrimination scores. For example, of

of lesion were generally consistent from frequency to frequency for all of the special tests in the *Menière's Disease + Other Cochlear Lesion* group given threshold levels that permitted valid ABLB and SISI testing. Different indications from frequency to frequency regarding site of lesion were found more often among the *Retrocochlear Lesion* and *Sudden Loss* groups.

Among the *Retrocochlear Lesion* group discrepancies between ABLB/SISI and Tone Decay/Békésy occurred in six instances (8%)—three "Yes–Yes" and three "No–No" as shown in Table 11 c In one of the "Yes–Yes" patients, recruitment was shown at the only testable frequency of 4 000 Hz, Tone Decay and Békésy were not tested at 4 000 Hz, but gave Type III patterns at all lower frequencies. In the other two "Yes–Yes" patients ABLB/SISI and Tone Decay/Békésy were done at the same frequencies.

For two of the three "No–No patients the special tests were given at the same frequencies. Reliability may have been poor for these two however because one spoke no English and was ill during the testing, and the other a 13-year-old child had to be tested quite rapidly because of scheduling demands. The latter had a meningioma in the cerebellopontine angle associated with von Recklinghousen's disease, and the former a sheath tumor described as rising from the Vth, rather than from the VIIIth nerve The third patient who had an acoustic neurinoma and a marginal pure-tone loss in the affected ear showed *no* recruitment at 250 Hz, the only frequency that allowed valid ABLB Her Békésy tracings were Type II at all frequencies, 250–4 000 Hz.

Inter test discrepancies occurred in six of the *Sudden Loss* patients (8) All six were Yes–Yes" (Table 11) In three of the six "Yes–Yes" patients, ABLB/SISI and Tone Decay/Békésy were done at the same frequencies, and in the remaining three, at different frequencies.

In summary then discrepancies between the two pairs of tests, although relatively infrequent for all groups, occurred more often in the *Retrocochlear Lesion* and *Sudden Loss* groups (both 8%) than in the *Menière's Disease + Other Cochlear Lesion* group (2%). Consequently in the absence of a history of sudden loss, such discrepancies suggest the likelihood of retrocochlear lesion. In any event the occurrence of these discrepancies, even though they involved only 16 (5%) of the total 333 patients, emphasizes the importance of doing at least one of the tests in each pair preferably at the same three or four frequencies. Testing with the remaining special tests would depend upon the reliability and definitiveness of results on the first two tests.

Ordinarily the frequencies in the 500- to 4 000-Hz range that showed the greatest pure tone loss provided the most useful information Interestingly four patients in the *Retrocochlear Lesion* group showed retrocochlear auditory signs in the form of Type III tone decay and significant Békésy shifts at only one frequency—2 000 Hz in every case.

Changes in Hearing

When special test results were inconsistent regarding site of lesion, or when other grounds for suspicion of retrocochlear involvement arose, the patient was generally *asked* to return in a month or two for a repetition of the tests (unless he underwent an operation performed on the basis of non auditory symptoms and signs) Suspicion might have been engendered for example by a "widening" pure-tone pattern even though special test results were cochlear by a parallel" pure tone pattern accompanying a minimal (10–20 dB) difference

Table 12. *Patients with brain-stem lesion*

Diagnosis	Pure-tone pattern	Ear	Pure-tone average (dB)	Speech discrimination (%)	Recruitment	Tone decay	Békésy theft
Brain-stem glioma	Widening	R	12	96 (78)	—		Insignificant
		L	23	100 (16)	—	I & II	
Brain-stem glioma	Widening	R	38	6	—	III	—
		L	12	100			
Brain-stem glioma	Widening	R	10	98 (68)			
		L	12	98 (50)	—	III	—
Brain-stem glioma	Widening	R	15	68	—	III }	Significant
		L	40	0	Present	III }	
Brain-stem glioma	Unclassifiable	R	15	90	—	III	Significant
		L	10	86			
Brain-stem glioma	Parallel	R	30	42	Absent	—	—
		L	5	100			
Multiple sclerosis	Normal	R	0	100 (50)			Significant
		L	2	96 (32)	—	—	
Multiple sclerosis	Normal	R	2	96	—	III }	Significant
		L	0	96	—	III }	
Multiple sclerosis	Closing	R	38	30	Absent	III	Significant
		L	20	94			
Multiple sclerosis	Widening	R	3	100			Significant
		L	18	64	Absent	III	
Cyst of the pons	Normal	R	12	100			Significant
		L	13	100	—	—	

Affected ear

( ) Speech discrimination distorted by noise.

results often enough that a strong prediction of a small tumor cannot be made in the individual case.

With respect to site of retrocochlear lesion, the large majority of tumors were reported to be in the cerebellopontine angle, and the point of origin was the VIIIth nerve within the internal auditory meatus. Constellations of special test results seemed generally no different, however, when the tumors involving the VIIIth nerve in the cerebellopontine angle arose from a different origin, such as the Vth nerve.

More promising were attempts to differentiate between the lesions localized primarily to the VIIIth nerve and other lesions localized primarily to the brain stem (comprising the mid-brain, pons, and medulla oblongata) (see Bunis, 1965). Table 12 presents the test results for 11 patients with lesions of the brain stem—six with brain-stem glioma, four with multiple sclerosis, and one with a pontine cyst.

Because the four patients with multiple sclerosis had lesions not verified surgically their inclusion in the "brain-stem lesion" group should be justified. It is known that many patients with multiple sclerosis have normal hearing for pure-tones (von Leden & Horton, 1948) and for tests of recruitment and tone decay (Dayal, Tarantino & Swisher 1966). When hearing impairment occurs, it may be manifest in various ways. Simpkins (1961) reported a characteristic pure-tone pattern in which the low tones were affected more than the high tones, but Dayal et al. (1966) found no such pattern. Recruitment is typically absent (Dix, 1965) tone decay may be present even with normal pure-tone hearing (Dayal, Tarantino & Swisher 1966). Finally all audiologic signs and symptoms may be completely reversible (Rose & Daley 1964 Dix, 1965).

Of our four patients with multiple sclerosis, one had a mild unilateral pure-tone loss with a

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1 In the brain-stem lesions of the present series, it seemed probable that either the cochlear nuclei were involved, or extensions involved the VIIIth nerve fibers in the cerebellopontine angle.

the 17 *Retrocochlear Lesion* patients who had cochlear test findings, 13 showed "widening" pure tone patterns three showed "closing both ends" patterns, and one showed a "closing" pattern A "widening pattern with cochlear results on the special tests occurred with a probability of 0 18 among the *Retrocochlear Lesion* patients, which was slightly greater than the probability of 0 13 among the *Menière s Disease + Other Cochlear Lesion* patients. Similarly a closing both ends" pattern with cochlear special test results occurred with a probability of 0 04 for the *Retrocochlear Lesion* group and with the same probability (0 04) for the *Menière s Disease + Other Cochlear Lesion* group Thus, the occurrence of a widening" or a "closing both ends" pattern despite coch lear special test findings suggests possible retrocochlear involvement. Even when non auditory symptoms and signs are absent, these patients should be followed carefully particularly when the history of onset is vague.

In other instances the pure-tone patterns may serve to increase confidence in the special test indications For example in only one instance (that of a patient with an arachnoid cyst) did a closing" pattern occur in combination with cochlear special test findings when a retrocochlear lesion was present On the other hand a "closing" pattern in combination with cochlear special test findings occurred in 73 of the 182 *Menière s Disease + Other Cochlear Lesion* patients. Similarly the "constricting pure-tone pattern in combination with cochlear special test findings occurred in 35 of the 182 patients with cochlear involvement and in none of the patients with retrocochlear involvement. Thus, cochlear involvement and the absence of retrocochlear involvement can be predicted with a high degree of confidence when a "closing" or constricting pure-tone pattern is combined with cochlear special test results.

The possible significance of a parallel" pure tone pattern occurring with a minimal dif ference in pure tone thresholds between the ears was discussed earlier Although these patterns alone should raise the question of possible retrocochlear lesion they of course achieve much greater significance when accompanied by Type III (or in some instances atypical) tone decay patterns, significant Békésy shifts, or marked reductions in speech discrimination

In an attempt to determine which inter test relations might predict type of lesion size of lesion, or specific site of lesion the test data and operative reports for the *Retrocochlear Lesion* patients were studied—with discouraging results. Operative reports were available for almost all of the 73 patients who had lesions involving the VIIIth nerve and who had taken the special tests. The data revealed no particular constellations of test results that could be correlated with type of lesion—that is, sheath tumor meningioma, cyst or brain-stem glioma.

Correlation attempts were also unrewarding with respect to size of lesion partly because information in the operative reports regarding tumor size was inadequate. It was evident, however that when all special test results indicated retrocochlear lesion large tumors sometimes accompanied mild pure-tone losses and small tumors sometimes accompanied severe losses, which suggests that tumor size cannot be pre dicted accurately from the pure tone level holding constant the special test results. A possible relation between size of tumor and consistency of the special tests in predicting retrocochlear lesion was suggested by Johnson (1968) He found that when some of the special tests pointed to cochlear rather than to retrocochlear involvement the tumor was often small Of our 17 patients in the *Retrocochlear Lesion* group whose special test results pointed to cochlear involvement ten had a sheath tu mor Of these ten, information regarding size of tumor was available for only seven all of whom had a moderate sensorineural loss in the affected ear The tumors were described in the operative reports as the size of a small lemon sizeable a large mass" moderate size" the size of a large pea 1 5 centimeters" and "one centimeter The first four descriptions seemed to indicate that large and moderate sized tumors produce inconsistent special test

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		L	40	0	Present	III}	
Brain-stem glioma	Unclassifiable	R	15	90	—	III	Significant
		L	10	86			
Brain-stem glioma	Parallel	R	30	42	Absent	—	—
		L	5	100			
Multiple sclerosis	Normal	R	0	100 (50)			Significant
		L	2	96 (32)	—	—	
Multiple sclerosis	Normal	R	2	96	—	III}	Significant
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		L	18	66	Absent	III	
Cyst of the pons	Normal	R	12	100			Significant
		L	13	100	—	—	

Affected ear.
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More promising were attempts to differentiate between the lesions localized primarily to the VIIIth nerve and other lesions localized primarily to the brain stem (comprising the mid-brain, pons, and medulla oblongata) (see Butts, 1965) Table 12 presents the test results for 11 patients with lesions of the brain stem—six with brain-stem glioma, four with multiple sclerosis, and one with a pontine cyst.

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closing" pattern and another had a moderate unilateral pure tone loss with a "widening" pattern. Both of these two patients showed 0% SISI scores, Type III tone decay significant Békésy shifts and no recruitment in the affected ear the better ear was normal on all tests. The other two patients (mentioned earlier) had normal and equal hearing for pure tones bilaterally One of these two showed significant fixed frequency Békésy shifts in one ear and no shifts in the opposite ear (tone decay was not done) The other showed Type III tone decay *and significant Békésy shifts bilaterally* Thus, the special test results for all four did indicate retrocochlear involvement—unilaterally in three and bilaterally in one.

Most investigators believe that the lesion causing hearing impairment in multiple sclerosis is in the area of the cochlear nuclei von Leden & Horton (1948) quote Leidler (1917) who in a series of 13 consecutive cases, found pathologic confirmation that the disease does have a predilection for the region of the vestibular and cochlear nuclei Dix (1965) states that the lesion must lie distal to the cochlear nuclei and central to the neurilemmal-neurologlial junction of the VIIIth nerve" The evidence was taken to indicate that patients with multiple sclerosis were appropriately placed in the brain-stem lesion" group

It seemed profitable to compare the results for these 11 patients (13 ears) with 20 patients studies by Parker et al. (1968) although the comparison is somewhat precarious considering the differences in classification of patients and in test materials and terminology For the sake of comparison, it must be agreed (1) that tone decay" is employed in the same sense as the "pathologic adaptation" of Parker et al., (2) that the Type III decay classification can be applied to severe tone decay by Carhart's (1957) test (see the data of Sorensen 1962) and (3) that the Parker pontine" group corresponds to our brain stem lesion" group Parker et al. did include *patients with multiple* sclerosis in their pontine" group On the other hand, they included several patients not represented in our group, namely patients with a diagnosis of "vertebrobasilar artery insufficiency" The findings of both series indicated that brain-stem lesion is suggested when Type III tone decay and significant Békésy shifts accompany normal or near normal pure tone hearing. The suggestion of brain stem lesion is stronger when Type III tone decay or significant Békésy shift occurs bilaterally

Speech discrimination findings showed good agreement in that about half the patients with brain-stem lesion in each group had scores of 80% or better In contrast scores for patients with VIIIth-nerve lesion were generally much lower in each group A score of 80% or better was made by only four of the 26 patients with VIIIth nerve lesion who formed the control group in the Parker series and by only three of our 17 patients with sheath tumors whose hearing levels were comparable to those of our brain-stem lesion" group—that is, thresholds under 40 dB Fletcherian average. Caution must be exercised in drawing conclusions regarding speech discrimination scores because all findings were based on live voice testing using different tests According to the data of Epstein Giolas & Owens (1968) we would expect the Harvard PB-50 lists (Parker series) to be somewhat more difficult than the W 22 lists (our series) under any circumstances of presentation

Substantial disagreement occurred only with regard to recruitment. Unfortunately the numbers for comparison are small. In our series, one in four of the "brain-stem lesion" patients who took the ABLB test showed recruitment, as did 17 of 61 (approximately one in four) other patients in the *Retrocochlear Lesion* group In the Parker series however five of the eight "pontine" patients who took the test showed recruitment in contrast to only three of 17 patients in their VIIIth nerve lesion group. Their conclusion that recruitment occurs frequently in patients with brain stem lesion runs counter to our findings and to observations of Dix (1965) who reported that recruitment is typically absent in multiple sclerosis.

It can be stated, on the basis of the two series, that brain-stem lesion, as opposed to VIIIth-nerve lesion, is suggested when Type III tone decay results and/or significant Békésy shifts (1) occur bilaterally (?) occur unilaterally along with normal or near-normal pure-tone hearing, or (3) occur with high speech discrimination scores (on the order of 80% or higher) on the W 22 lists. The suggestion of brain-stem lesion as opposed to VIIIth-nerve lesion appears to be strongest in (1) and weakest in (3)

The relation between Tone Decay/Békésy and speech discrimination deserves mention. In our *Retrocochlear Lesion* group Type III tone decay or significant Békésy shifts in the speech frequency range were generally accompanied by extremely low speech discrimination scores, although high scores occurred on occasion. Extremely low discrimination scores were found infrequently in the absence of Type III tone decay or significant Békésy shifts. Thus the Tone Decay and Békésy (fixed frequency) tests seem to be more sensitive to retrocochlear involvement than the speech discrimination scores, at least insofar as the VIIIth-nerve and brain-stem lesions are concerned.

Reports by Goetzinger & Angell (1965) and Hodgson (1967) suggest that with cortical lesion, reduction in speech discrimination may be the more sensitive indicator That is, they found reduced discrimination on difficult tests (as by frequency distortion) on the side opposite to a cortical lesion. Neither tone decay nor Békésy shifts were present. One could speculate whether Tone Decay/Békésy results might be significant with cortical lesion were these tests also made more difficult, for example, by adopting a two- or three-minute, *rather than* a one minute, criterion for tone decay

Discussion

Comments on ABLB

Of major concern is that the special tests pointed to cochlear involvement in 22% of patients with known retrocochlear lesion. This percentage is in contrast to the 10 reported by Dix & Hallpike (1960) for the ABLB test alone and the approximately 40% reported by Johnson (1968) for a test battery similar to that of the present survey Johnson's data indicated that a substantial proportion of the patients in this 40% showed inter-test inconsistencies, which might have been interpreted as suggestive of retrocochlear involvement in light of the inter-test consistency of the cochlear lesion patients in the present survey and the consistency on ABLB shown by patients with Meniere's disease reported by Hood (1969).

No doubt the differences in results for the three studies are at least in part a function of the populations involved. Johnson felt that the less positive auditory findings for his 1968 series compared to an earlier series "may be partially explained on the basis of earlier detection of acoustic neuroma before the cochlear branch of the eighth nerve is fully involved" With respect to Hood's population, the cochlear group consisted entirely of patients with Menière's disease, a relatively clear-cut cochlear entity Slightly greater uncertainty regarding site of lesion may be encountered in patients with other kinds of cochlear impairment and in some patients with a history of sudden loss.

Hood (1969) in his work with Dix & Hallpike, placed reliance primarily on the *ABLB* test in *differentiating* between cochlear and retrocochlear lesions. If we had depended only upon the ABLB *test*, however we would have failed to disclose audiometric evidence of retrocochlear involvement not only in the 17 pa-

tients for whom all special test results indicated cochlear involvement, but in four others for whom ABLB indicated cochlear involvement while Tone Decay/Békésy indicated retrocochlear involvement. Also valid ABLB testing was precluded in six of our 78 patients with retrocochlear lesion in whom retrocochlear involvement was indicated by the Tone Decay and Békésy tests. Three of the six had normal and equal pure tone thresholds bilaterally one had a bilateral high-tone loss another was a seven-year-old child who could not perform reliably on the ABLB and the sixth had a reversal in pure-tone thresholds—that is the ear affected by the tumor showed better thresholds than the opposite ear which had been affected primarily by exposure to noise. If to these six patients we add the 21 above we would have failed to disclose audiometric evidence of retrocochlear involvement in 27 instances (35%) with only ABLB Also we found a few patients who experienced much difficulty in the loudness balancing task and a few others whose severe losses precluded completion of the ABLB (The SISI test often served nicely in these instances) Clearly either the Tone Decay or Békésy test is necessary for a thorough investigation of possible retrocochlear involvement. In fact, if the audiologist were limited to one test, he would be wiser to select the Tone Decay or Békésy (fixed frequency) because either can be used with validity for all but the most severe losses Of these two tests the Békésy would probably be the better choice because the Tone Decay test seems slightly more prone to artifact. Using Tone Decay/Békésy alone, we would have failed to disclose retrocochlear involvement in 20 patients (26%) of the Retrocochlear Lesion group—that is in the 17 exceptional patients mentioned earlier plus three others who showed neither recruitment nor significant Tone Decay/Békésy results.

Hood would no doubt suggest that ABLB should have been done to explore for possible "loudness reversal"[1] in four of the six patients mentioned above namely the three with normal and equal hearing for pure tones bilaterally and the one with a bilateral high-tone loss. ABLB was done in only one of the three patients with normal and equal pure tone hearing bilaterally and no abnormality of loudness growth was found. The importance of ABLB in such cases is not denied but loudness reversal will not be found in all instances. Should loudness reversal (decruitment) be demonstrated, however information from Tone Decay/Békésy would still be needed. For example abnormally slow loudness growth has been shown in the ear contralateral to a cortical lesion (Jerger 1960 b Davis & Goodman, 1966 Hodgson, 1967) but in such a lesion the affected ear has shown no significant Békésy shifts (Goetzinger & Angell 1965 Hodgson 1967) Thus the occurrence of Type III tone decay or significant Békésy shifts on the same side as the loudness reversal might suggest a retrocochlear lesion lower in the auditory system as opposed to cortical involvement.

The marked discrepancy between Hood's (1969) and our results for ABLB called for a review of our findings Except for our portraying the loudness matches on laddergrams instead of graphs, our ABLB procedure for 70% of the patients (those in whom the reference tones for the loudness matches were fixed in the better ear) was not unlike the one described by Hood in 1969 His comments, however were directed to the Jerger (1962) method of fixing the reference tone in the poorer ear which method we followed in 30% of our patints Hood was concerned that Jerger's method might tend to *hide* recruitment where it is actually present thus suggesting a retrocochlear lesion that does not exist. It is interesting that our problem was the reverse. recruitment often camouflaged an existing retrocochlear lesion It should be stressed that in the patients for whom the reference tone was

[1] Hood and his co-workers have employed the term loudness reversal" for the abnormally slow growth of loudness in the affected ear More recently Davis & Goodman (1966) have employed the term decruitment for the same phenomenon.

fixed in the poorer ear our approach was a manual one that did not follow the complete protocol offered by Jerger (1962)

For the *Menière's Disease + Other Cochlear Lesion group* 80 laddergrams were carried to completion "fixing" the better ear at 20 dB intervals. Recruitment was complete in 74 patients, and partial, but clearly present, in six. In 29 other patients fixing the better ear the laddergram was not completed because recruitment was immediately evident after two or three matches, or recruitment was reported in the records, but the laddergrams were not available for analysis.

In 48 patients from the *Menière's Disease + Other Cochlear Lesion* group, the reference tone was fixed in the poorer ear Following Jerger's (1962) definitions, recruitment was found to be complete in 33 and partial in 15 patients.

In the *Retrocochlear Lesion* group, excluding the 17 exceptional patients mentioned earlier 24 laddergrams fixing the better ear were carried to completion. 23 showed no evidence of recruitment and one showed complete recruitment at the only testable frequency (4 000 Hz). The affected ear was fixed in 18 patients. 14 showed no evidence of recruitment, three showed partial recruitment at one frequency and no recruitment at other frequencies and one showed partial recruitment at several frequencies.

For the 17 *Retrocochlear Lesion* patients whose special test results indicated cochlear involvement, laddergrams fixing the better ear were carried to completion in nine: eight showed complete recruitment, and one showed partial recruitment In a tenth patient, the laddergram was not completed because recruitment was immediately obvious. In four other patients, the poorer ear was fixed. complete recruitment was shown in one, partial recruitment in three. For the remaining three of the 17 patients, ABLB testing was not feasible in one and was omitted in the other two (in these two, SISI scores were 100 %) In retrospect, ABLB results for two of the 17 patients were of special interest. Both showed recruitment at one testable frequency but not at others. These results occurred in three of the *Retrocochlear Lesion* group for whom retrocochlear lesion was indicated on the basis of Tone Decay/ Békésy results, but in none of the *Menière's Disease* group and in only two of the *Other Cochlear Lesion group*. Consequently such results should strongly suggest the possibility of retrocochlear lesion. Obviously if feasible ABLB testing should be done for at least two frequencies.

It was readily evident that a higher proportion of "partial" recruitment results occurred when the reference tone was fixed in the poorer ear probably in part because only one loudness match (at 20 dB SL) was obtained in the majority of cases. Although the ABLB results generally supported the common policy of associating partial recruitment with cochlear involvement, the fact that partial recruitment occurred occasionally in the *Retrocochlear Lesion* group suggests the advisability of carrying out the test until the whole picture of recruitment can be seen, regardless of which ear is fixed. Hood (1969) in this connection reported that in a series of 424 patients with Menière's disease, all showed recruitment, and in 415 the recruitment was complete. This left only 9 (2 %) showing partial recruitment. Partial recruitment, by itself should not be considered a strong indicator of cochlear involvement.

Since the data of this survey are insufficient to weigh adequately the merits of fixing the good versus the bad ear the matter will not be pursued. An appeal of the Hood approach to ABLB however lies in his report that even when loudness recruitment occurs with VIIIth nerve lesions, the majority of recruitment curves differ conspicuously from those for patients with Menière's disease. He maintains "that these small but clinically significant features can only be revealed if (*a*) a systematic series of balancing points is established at intervals of 20 dB in the good ear and (*b*) the results are graphically displayed and not transferred to ladder diagrams A pertinent question, of

course, is whether the recruitment curves of Menière's disease patients can be generalized to patients with other kinds of cochlear impairment.

In any event, we agree with Hood that the primary need is for careful testing by a skilled and resourceful audiologist In the population suspected of possible retrocochlear involvement the audiologist must often work against the patient's anxiety fatigue or illness, and must often obtain maximum information in a minimum of time. Testing time can be reduced in many instances For example, consider a patient with a history suggestive of Menière's disease, a closing" or "constricting" pure tone pattern and W 22 scores generally proportionate to the pure tone levels If complete loudness recruitment or high SISI scores are demonstrated at two or three frequencies, the testing can usually be concluded satisfactorily by a screening for tone decay or by a few fixed frequency Békésy tracings Similarly consider a patient with a vague history of onset the likelihood of progressive loss a widening" pure tone pattern and an extremely low W 22 score in the face of a mild pure-tone loss in the affected ear Significant fixed frequency Békésy shifts or Type III tone decay at a few frequencies should be sufficient to report evidence of retrocochlear involvement. An absence of recruitment, or low SISI scores would add strength to the evidence. In other words, completion of all four special tests in the above examples would typically yield no additional information clinically speaking, with regard to the question of cochlear versus retrocochlear involvement. Usually the need for further testing is indicated by the definitiveness and consistency of the first two special tests that are employed—for example, the ABLB and Tone Decay ABLB and Békésy SISI and Tone Decay or SISI and Békésy

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3 Pure-tone patterns for unilateral loss patients were classified according to the relation between the configurations for the two ears, thus taking into consideration the prevalence of better-ear high-tone loss in all groups. Parallel" patterns were found in all groups, but relatively less often in the *Retrocochlear Lesion* group Constricting" patterns occurred almost exclusively in the *Menière's Disease* and *Other Cochlear Lesion* groups. Closing" patterns predominated in the *Menière's Disease* and *Other Cochlear Lesion* groups they occurred frequently in the *Sudden Loss* group although only with greater degrees of impairment, and they were infrequent in the *Retrocochlear Lesion* group. Widening" patterns were characteristic of the *Retrocochlear Lesion* group, but they

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4. When discrimination scores on the W 22 lists fell below 48% with thresholds of less than 40 dB for the Fletcherian pure-tone average, retrocochlear involvement could be predicted with high confidence. The only exceptions were in elderly patients or patients with a sharply downward sloping pure-tone configuration in the speech frequency range. Depending upon the pure-tone hearing level, scores both lower and higher than 48% offered clues in predicting retrocochlear lesion.

5. Special tests (ABLB, SISI, Tone Decay and Békésy) were given to 170 of the *Menière's Disease* group, 62 of the *Other Cochlear Lesion* group 78 of the *Retrocochlear Lesion* group, and 73 of the *Sudden Loss* group. Evidence suggested that when two tests—one of each pair (*ABLB SISI* and *Tone Decay Békésy*)—provided definitive results, further testing typically added no information with respect to cochlear versus retrocochlear lesion. In the *Menière's Disease* and *Other Cochlear Lesion* groups combined, four patients, two in each group showed mixed cochlear-retrocochlear signs. The remaining 178 patients (97.8%) with cochlear lesions showed cochlear signs. In the *Retrocochlear Lesion* group, 53 patients (68) showed retrocochlear signs, 17 (%) showed cochlear signs, and eight (10%) showed mixed cochlear retrocochlear signs. In the *Sudden Loss* group, 55 patients (75%) showed cochlear signs, ten (14%) showed retrocochlear signs, and eight (11%) showed mixed cochlear-retrocochlear signs.

Accordingly in the absence of a history of sudden loss, mixed cochlear-retrocochlear signs suggest the likelihood of retrocochlear involvement. These mixed signs usually refer to discrepancies between the two pairs of tests—ABLB/SISI versus Tone Decay/Békésy—regarding site of lesion, and emphasize the importance of doing at least one of the tests in each pair preferably at the same three or four frequencies.

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7. Inconsistent, unusual, or otherwise suspicious test results regarding site of lesion can often be resolved upon retesting after a few months. The following are apt to occur with retrocochlear involvement. (*a*) an increase in pure-tone hearing loss (although there seems no lawful pattern of progression with respect to the frequency range affected) (*b*) a reduction in speech discrimination that is sometimes markedly out of proportion to pure-tone changes and (*c*) changes in special test results with respect to site of lesion.

8. Observation of the constellations of test results suggested the following: (*a*) Retrocochlear involvement should be reported as a possibility with "widening" or "closing both ends" pure-tone patterns, even when they appear with cochlear special test findings, and especially when the history of onset is vague. (*b*) Cochlear involvement, and the absence of retrocochlear involvement, can be predicted with a high degree of confidence for a given individual

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when cochlear special test results are combined with bilateral loss or with a "closing" or "constricting" pure-tone pattern (*c*) Brain-stem lesion as opposed to VIIIth-nerve lesion, is suggested when Type III tone decay patterns and/or significant Békésy *shifts (1) occur bilaterally* (2) occur unilaterally with normal or near normal pure tone hearing, or (3) occur with high discrimination scores 80% or higher on the W 22 lists The suggestion of brain stem lesion is probably strongest in (1) weakest in (3) (*d*) Meaningful correlations could not be *found* between particular test constellations and type or size of retrocochlear lesion.

Conclusions

For the group of patients described here, who were referred to the audiology clinic on suspicion of retrocochlear hearing impairment the following conclusions may be drawn

1 The ABLB SISI Tone Decay and Békésy hearing tests are invaluable adjuncts to the basic pure tone and speech reception tests. If certain discrepancies between the tests are included as indicators of retrocochlear involvement, such involvement should be correctly predicted in at least 78% of the cases.

2 On the other hand, the failure of all four tests to correctly predict approximately one out of five retrocochlear lesions (22%) indicates that caution is necessary in interpretation, and that other audiometric clues must be observed

3 The pure-tone findings are definitely helpful in predicting retrocochlear lesion and may serve to reduce the failure rate of the special tests.

4 Extremely low speech-discrimination scores may provide a strong indication of retrocochlear involvement, especially in cases where pure tone impairment is relatively mild

5 Because of the general consistency and definitiveness of the four special tests in the patients with cochlear lesion, the occurrence of any unusual or inconsistent findings should alert the audiologist to the possibility of retrocochlear involvement. In such cases, retesting at a later date often produces definitive information.

6 Limitations and possible artifacts are present in all four special tests, and one or more may fail to provide reliable and valid results for a given patient. Assuming good reliability however the ABLB and the SISI tests typically point to the same conclusion regarding cochlear versus retrocochlear lesion as do the Tone Decay and Békésy tests.

7 Certain constellations of test results including pure-tone findings and speech-discrimination scores may strengthen prediction of cochlear or retrocochlear involvement they may help in differentiating between VIIIth-nerve and brain-stem involvement and they may help in distinguishing between hydrops and other kinds of cochlear involvement. (Lesions of the higher auditory pathways and cortex were not included in this survey)

8 Accurate history of the onset of hearing impairment is important, because patients who experience sudden loss often show the same pure tone patterns, speech-discrimination results, and special test results as patients with retrocochlear lesions, although no lesions at least in the form of neoplasms, have been found in our *Sudden Loss* patients to date

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SUPPLEMENT 234

Depression of the Click Action Potential by Attenuation, Cooling, and Masking

BY

A. C. COATS

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SUPPLEMENT 284

Depression of the Click Action Potential by Attenuation, Cooling, and Masking

BY

A. C. COATS

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Baylor College of Medicine, and the Methodist Hospital, Houston, Tex., USA

UPPSALA 1971

Introduction

The experiments reported herein compare the effects of cooling, attenuation of test click, and physiological masking on the auditory-nerve action-potential response to clicks (abbreviated AP). By using averaged APs the effects of the three treatments on AP wave form could be compared much more confidently than is possible with photographs of single oscilloscope traces.

In this report, physiological masking is defined as the reduction in AP amplitude which is observed when white noise, or a pure tone of low-to-moderate intensity is applied along with the click (Coats, 1967). With physiological masking, the click microphonic is unchanged, and the term, as used in this report, excludes the effects of high-intensity stimuli where microphonic depression is observed.

Methods

Recording and data processing

APs were recorded from the round window and auditory nerve of the cat. A silver-ball electrode was used to record from the round window and a coaxial electrode was used to record from the auditory nerve. All of the results presented were confirmed with recordings from both locations.

Two different methods of data analysis were used. In earlier experiments, we photographed oscilloscope records of the click APs and took measurements from the photographs. More recently we recorded the APs on magnetic tape and averaged them off-line with a LINC-8 computer.

Fig. 1 shows a block diagram of the recording and data-processing system. The preparation was in an electrically shielded, sound-isolating room; the electrodes were led to a differential preamplifier (Tektronix, type 122), with the upper 3-dB point set at 10 kHz and the lower 3-dB point at 80 Hz.

The output of the preamplifier was led to one channel of an analog tape recorder (Precision Instruments) and was also monitored by an oscilloscope. The electrical square wave, which drove the earspeaker to produce the click stimulus, was recorded on a second tape channel and served as a trigger pulse to start analog-to-digital conversion. A third channel contained a "level code" (±1 V d.c.) which was used to signal the end of one run and the beginning of another. The level code and APs were recorded at 60 inches/sec in the FM mode, giving a frequency response (±3 dB) of 0–20 000 Hz; the trigger pulse was recorded in the direct mode, giving a frequency response (±3 dB) of 100–20 000 Hz. The analog signal was reproduced into the LINC-8 analog-to-digital converter at 7 $^1/_2$ inches/sec, thus multiplying the effective conversion rate by 8.

The computer processed the data in two steps: (1) analog-to-digital conversion, and (2) averaging. Utility programs included a plotting program and a program for editing digital-data tapes.

The analog signal was converted into 8-bit

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[Gen]eration of acoustic stimuli

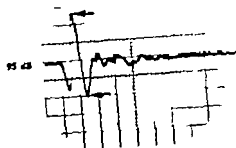
[Fig]. 2 diagrams the instrumentation for gener[at]ing acoustic stimuli. Two electrically shielded [P]DR-600 earspeakers were mounted about 2 [cm] from the severed end of the external audi[to]ry canal. The masking stimuli were pro[du]ced by driving one of the earspeakers with the [o]utput of either a white-noise or a sine-wave [g]enerator. Driving the second earspeaker with [0].01 msec square-wave pulses produced the [c]licks. Decade attenuators calibrated in deci[b]els controlled sound intensity.

A weighted sound-intensity measurements were made with a probe-tube microphone which had been damped by packing with steel wool to make its frequency response as linear as possible. The probe tube was placed next to the severed end of the external canal (perpendicular incidence). The measurements were checked at the end of the experiment by removing the preparation and mounting a condenser microphone where the external canal had been.

All intensity measurements are expressed in sound-pressure level (dB re 0002 μbar). Click intensities are expressed in "peak-equivalent" dB, SPL. Fig. 3 shows the wave form of the click and the points used to determine peak to-peak amplitude. With the ear of a human observer placed approximately in the position occupied by the severed end of the animal's external canal, the click threshold was 25–30 dB peak-equivalent SPL. The "visual-detection level" of the *averaged* AP was generally about 5 dB below the human threshold. The visual-detection level of individual APs was at or slightly above human threshold.

Preparation of experimental animals

The cats were anesthetized with an intraperitoneal injection of Nembutal, and a d.c. heat lamp maintained body temperature during the experiment. A posterior approach to the bulla exposed the round window and, when nerve recordings were made, a dorsal approach (which included sucking out overlying cerebellum) ex-





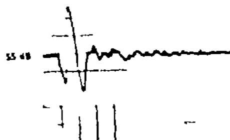


Fig. 3. Click stimuli at different intensities demonstrating linearity over the range studied. A 1-inch B & K condenser microphone (parallel incidence) located 2 inches from the speaker picked up the clicks. The frequency response of the sound level meter was linear over the 20–20 000 Hz range. Click intensities are given in peak-equivalent SPL. Arrows show the peaks used to determine peak-equivalent values. As we increased the click intensity we attenuated the input to the sound-level meter by an equal amount. Five superimposed traces are photographed. The time scale is 0.2 msec per division.

RECORDING AND DATA PROCESSING

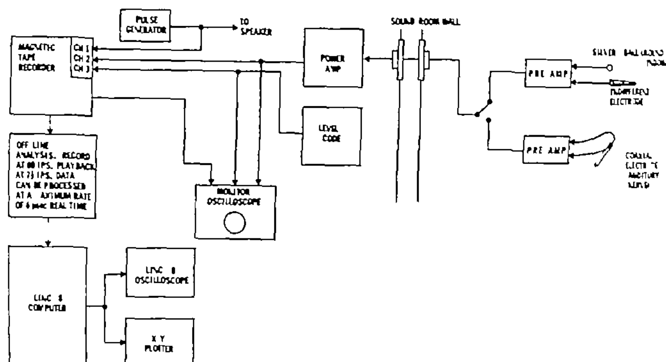


Fig 1 Block diagram of the recording and data-processing system used to obtain averaged cochlear microphonic and AP response to click stimuli.

binary words, giving a range of 511 voltage "steps" The time between successive samples could be preset from 5 6 μsec to 478 μsec (real time) and the number of samples could be preset from 256 to 1 240 We routinely used a real time conversion rate of 9 μsec per sample, with 512 samples per AP

GENERATION OF ACOUSTIC STIMULI

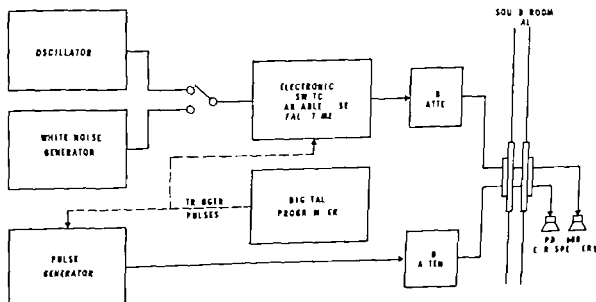


Fig 2 Instrumentation for generation of acoustic stimuli.

Generation of acoustic stimuli

Fig. 2 diagrams the instrumentation for generating acoustic stimuli. Two electrically shielded PDR-600 earspeakers were mounted about 2 cm from the severed end of the external auditory canal. The masking stimuli were produced by driving one of the earspeakers with the output of either a white-noise or a sine wave generator. Driving the second earspeaker with 0.01 msec square-wave pulses produced the clicks. Decade attenuators calibrated in decibels controlled sound intensity.

A weighted sound-intensity measurements were made with a probe-tube microphone which had been damped by packing with steel wool to make its frequency response as linear as possible. The probe tube was placed next to the severed end of the external canal (perpendicular incidence). The measurements were checked at the end of the experiment by removing the preparation and mounting a condenser microphone where the external canal had been.

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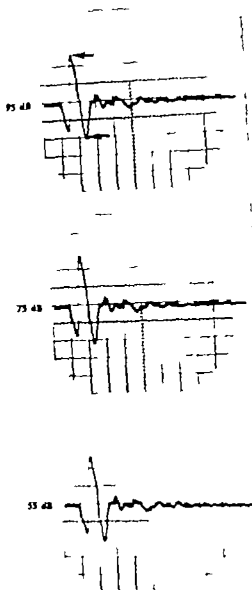


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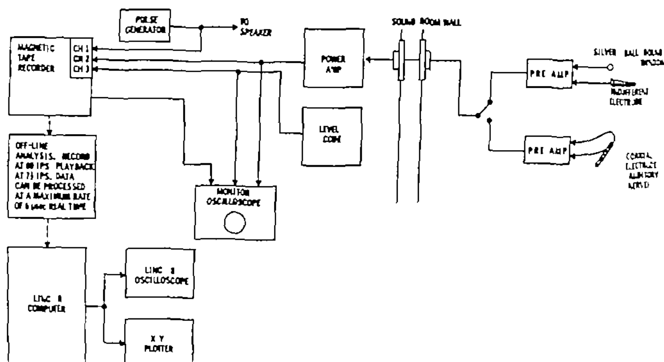


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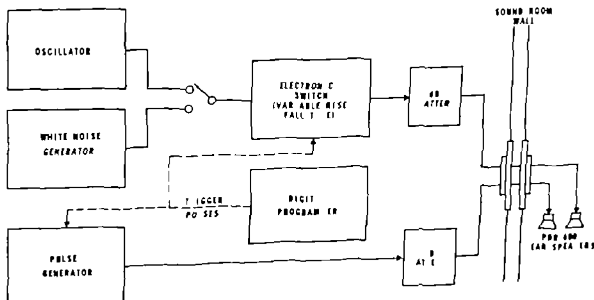


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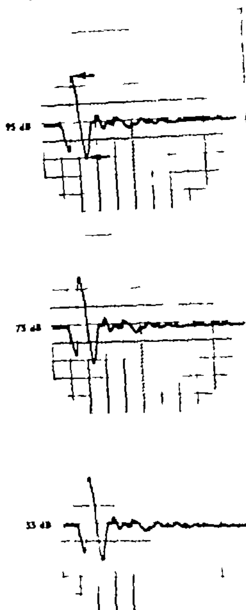


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posed the auditory nerve All experiments, whether recordings were taken from nerve or round window were done with the bulla open. In all preparations the pinna was removed, leaving about a 3 mm stump of external canal.

In several experiments we cut the middle-ear muscles. Under microscopic observation the bony septum was removed with fine scissors and a rongeur A final widening of the opening was made with a dental drill, but we used the drill sparingly in order to avoid, insofar as possible acoustic trauma by bone-conducted sound

In all preparations with middle-ear muscles cut, we were careful to avoid accumulation of fluid in the middle-ear space All the reported results were obtained with middle-ear muscles cut and with them intact.

Application of localized temperature change

A thermoelectric cooling device, consisting of a bimetallic junction with one side equalized with running water and the other side thermally coupled to (but electrically insulated from) a silver rod 6 cm long, controlled the temperature in the vicinity of the cochlea. Teflon shink-fit" tubing insulated the rod, whose tip was bent upward about 30 and was flattened to provide a contact area of about 3 mm² Temperature at the tip of the rod was monitored with a calibrated thermocouple. A micromanipulator held the instrument and positioned it so that the flat tip of the rod rested firmly against the bony ledge beneath the round window

Results

Effects of masking, cooling and attenuation on AP latency and wave form

In five preparations AP amplitude was reduced equally by masking, cooling, and attenuation. All preparations were stimulated with 70–85 dB (peak equivalent) control clicks. Two were also stimulated with 60 dB control clicks. In each experiment, masking intensity and amount of cooling and attenuation were adjusted to produce about 50% reduction in N_1 amplitude

In Fig. 4 click APs equally reduced by attenuation, cooling, and masking are shown (plotter gain adjusted to produce equal amplitude so that wave forms can be compared) As illustrated by these records, cooling and attenuation always produced equal latency[1] increases, and the wave forms of the cooled and attenuated APs were nearly identical. (Small inconsistent differences in relative amplitudes of N_1 and N_2 were, however sometimes observed)

The records in Fig. 4 also demonstrate that, in comparison with cooling and attenuation, *masking has relatively little effect on* AP latency These records are, however atypical in that the slight latency increase usually observed with masking is not present. In this series, this was the only instance in which masking failed to produce a latency increase but a critical review of our earlier photographic records uncovered a few examples of either a failure to increase latency or a questionable latency increase. This apparent variability prompted the following additional study of the effect of masking on latency

[1] In all results reported here, "latency" refers to the interval between the electrical pulse producing the click and the peak of N_1 This includes sound-conduction time, which was about 60 μsec. Amplitude was measured from the first negative AP peak (N_1) to the positive peak between N_1 and N_2

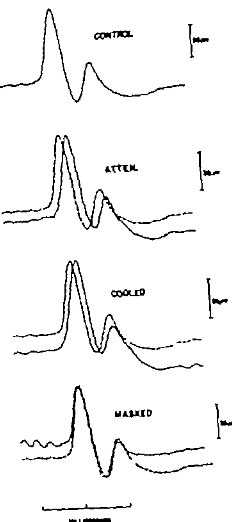


Fig. 4. Averaged click APs reduced equally by masking, cooling, and attenuation. As in all records shown in this report, upward deflection represents negativity at the round window. To facilitate waveform comparison, we adjusted the gain so that all tracings show equal N amplitude. The control click is retraced as dashed line onto the attenuated, cooled, and masked AP tracings. Each tracing is an average of 50 APs. The control-click intensity was 59 dB, peak equivalent SPL, and we attenuated the click by 9 dB to produce the second tracing. The cold probe, set at 12.5°C, produced the third tracing, and to produce the bottom tracing we applied white-noise masking stimulus of 30 dB, SPL.

Effect of intensity on latency change produced by masking

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In all the previous instances in which masking appeared to have an insignificant effect on latency, click intensity was either low or moderate, and masking intensity was relatively high. Thus, the present results seem to identify the source of variability of the effect of masking on AP latency.

Throughout these experiments we consistently observed that masking, attenuation, and cooling not only increased the latency of the N peak, but also slightly broadened it. The effect

posed the auditory nerve All experiments, whether recordings were taken from nerve or round window were done with the bulla open. In all preparations the pinna was removed leaving about a 3-mm stump of external canal.

In several experiments we cut the middle-ear muscles. Under microscopic observation, the bony septum was removed with fine scissors and a rongeur A final widening of the opening was made with a dental drill, but we used the drill sparingly in order to avoid, insofar as possible, acoustic trauma by bone-conducted sound

In all preparations with middle-ear muscles cut, we were careful to avoid accumulation of fluid in the middle-ear space. All the reported results were obtained with middle-ear muscles cut and with them intact

Application of localized temperature change

A thermoelectric cooling device consisting of a bimetallic junction with one side equalized with running water and the other side thermally coupled to (but electrically insulated from) a silver rod 6 cm long, controlled the temperature in the vicinity of the cochlea. Teflon "shink fit" tubing insulated the rod, whose tip was bent upward about 30 and was flattened to provide a contact area of about 3 mm². Temperature at the tip of the rod was monitored with a calibrated thermocouple A micromanipulator held the instrument and positioned it so that the flat tip of the rod rested firmly against the bony ledge beneath the round window

Results

Effects of masking, cooling and attenuation on AP latency and wave form

In five preparations AP amplitude was reduced equally by masking, cooling, and attenuation All preparations were stimulated with 70–85 dB (peak equivalent) control clicks. Two were also stimulated with 60 dB control clicks. In each experiment, masking intensity and amount of cooling and attenuation were adjusted to produce about 50% reduction in N_1 amplitude

In Fig. 4 click APs equally reduced by attenuation, cooling, and masking are shown (plotter gain adjusted to produce equal amplitude so that wave forms can be compared) As illustrated by these records, cooling and attenuation always produced equal latency[1] increases, and the wave forms of the cooled and attenuated APs were nearly *identical*. (Small, inconsistent differences in relative amplitudes of N_1 and N_2 were, however sometimes observed)

The records in Fig. 4 also demonstrate that, in comparison with cooling and attenuation, masking has relatively little effect on AP latency These records are however atypical in that the slight latency increase usually observed with masking is not present. In this series, this was the only instance in which masking failed to produce a latency increase but a critical review of our earlier photographic records uncovered a few examples of either a failure to increase latency or a questionable latency increase. This apparent variability prompted the following additional study of the effect of masking on latency

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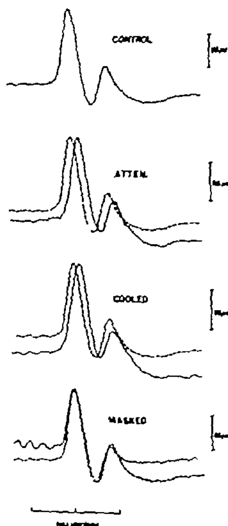


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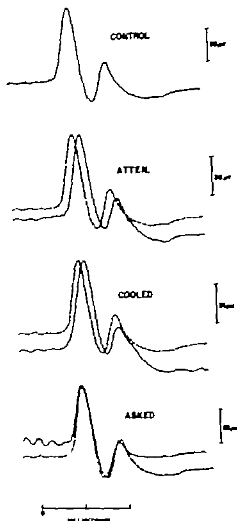


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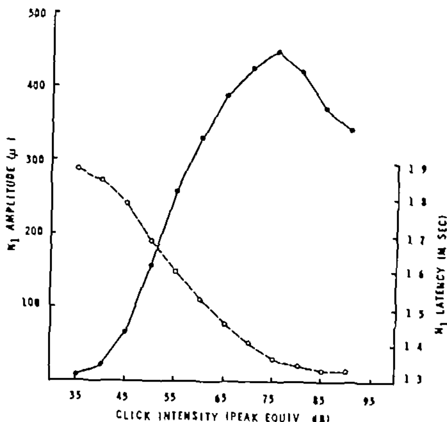


Fig 5 A latency (– –) and amplitude (——) plotted against click intensity

is small unless reduction in AP amplitude is quite large (at least 75%) Figs. 7 and 8 demonstrate this observation. The records shown in these figures were obtained by adjusting plotter gain to produce equal N_1 amplitudes and positioning so that the N_1 peaks coincide.

Effect of masking frequency on latency change produced by masking

Five preparations were masked with pure tones and three preparations with one-third-octave, band-passed white noise Each experiment tested frequencies ranging from 0 5 kHz to 12 kHz. Throughout each experiment the acoustic stimuli were monitored with a calibrated probe tube to be certain that there was no acoustic transducer overload at high intensities.

Masking at various frequencies produced no differential effects on masked AP latency This is illustrated by Figs. 9 and 10 Fig. 9 shows plots of masked amplitude and latency versus masking intensity for a series of masking frequencies (pure tones) These plots are equivalent to those in Fig. 6, except the latency scale has been expanded. It appears that for a given reduction in AP amplitude, masking produces the same latency increase regardless of masking frequency Fig. 10 shows this more directly In this figure, amplitude change (unmasked amplitude minus masked amplitude) is plotted against latency change (masked latency minus unmasked latency) The plots for different masking frequencies appear superimposable.

Latency change during onset of and recovery from masking

Four preparations were studied, two with the middle-ear muscles cut and two with them intact. In two preparations (one with and the other without middle-ear muscles cut) pure-tone and white noise masking yielded qualitatively similar results. The other preparations were masked only with white noise. We chose masking and test-click intensities to produce a maximal latency increase The rise-fall time of the masking stimulus was 7 5 msec Onset

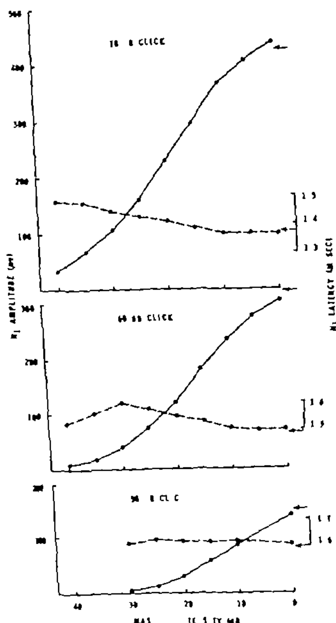


Fig. 6. N latency (– –) and amplitude (——) plotted against masking intensity (white noise) at three different test-click intensities. *This is the same preparation* show in Fig. 5 Arrows at the right show unmasked amplitudes. The masking-intensity scale has been inverted to facilitate comparison with the click AP input-output curve (Fig. 5).

and cutoff were smooth and reasonably free of echo (see Fig. 11). We obtained averaged responses to single clicks delivered at various times during and after cutoff and onset, and plotted onset and recovery curves for amplitude and latency

Typical results are shown in Figs. 12 and 13 For these figures, the latency scale is inverted to allow comparison of the latency and amplitude plots. As shown, latency follows amplitude *fairly closely* *both during the onset* and after the cutoff of the masking stimulus. The only possible exception is during the first few milliseconds of masking onset, where the latency increase appears to lag the decline in amplitude. This apparent dissociation appear

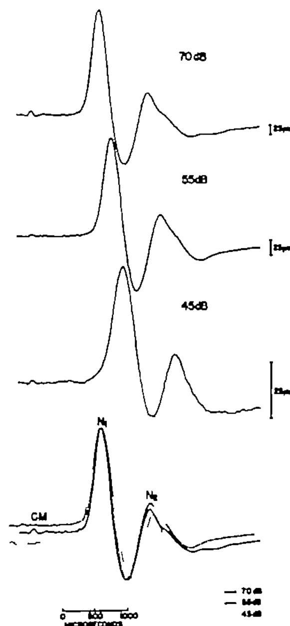


Fig 7 Effect of attenuation on the wave form of *N* Each tracing is an average of 70 responses (gain adjusted to produce equal *N* amplitude) Click intensity shown at the right, is in peak-equivalent decibels. At the bottom, the action potentials are retraced and positioned so that the *N* peaks coincide. The components of the round-window response are labeled *CM* – cochlear microphonic response to click. *N* = first negative whole-nerve AP peak. N = second negative AP peak. This is the same preparation shown in Figs. 5 and 6

ed in one of the other preparations but not in the remaining two We observed that at low masking intensities amplitude reduction is sometimes relatively greater than latency increase Therefore, this apparent dissociation between amplitude and latency during the first 2–3 msec of masking onset could be attributed to an intensity effect.

As a result of these experiments we conclude that there is no significant dissociation between either the time course of decline in AP amplitude and increase in latency at masking onset or the increase in AP amplitude and decrease in latency during recovery

Effect of temperature on masking recovery

We have previously reported that increasing masking duration does not change masked AP amplitude but does slow the recovery rate (Coats, 1964 *a*) In contrast, increasing masking intensity reduces masked amplitude but

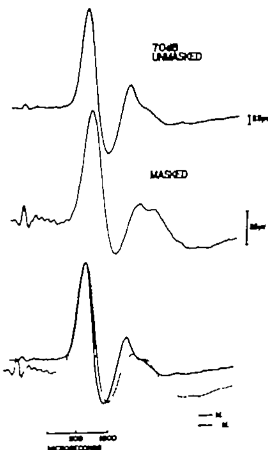


Fig 8 Effect of masking on the N wave form This is the same preparation shown in the preceding three figures. The middle-ear muscles were cut, and the masking stimulus was white noise at 38 dB SPL.

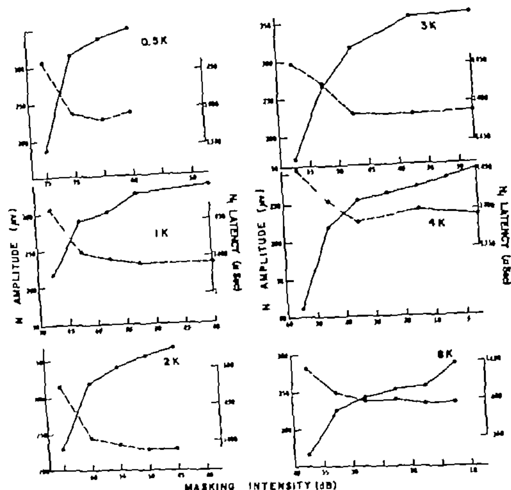


Fig. 9. N_1 latency (- -) and amplitude (——) plotted against masking intensity at six different masking frequencies (middle-ear muscles cut). Here, the masking stimuli were pure tones. Test-click intensity was 55 dB (peak equivalent). The *masking-intensity* scales have been inverted. Each data point was taken from an average of 30 round-window responses.

recovery from masking at different intensities (constant duration) follows a parallel time course (Coats, 1964 *b*). The effect of changing test-click intensity was equivalent to the effect of changing masking intensity. The experiments reported here were undertaken to determine whether the effect of temperature more closely resembles the effect of masking duration or of stimulus intensity.

Four preparations were masked with white noise. We cut the middle-ear muscles of two. Two preparations (one with and one without cut middle-ear muscles) were masked with a 500-Hz tone as well as with white noise. We applied each masking stimulus several times, and after each cutoff delivered a train of clicks (click rate = 20/sec). To plot recovery, we averaged every first AP, every second AP, and so forth.

The results, illustrated by Figs. 14 through 17, were essentially the same for all experiments. As shown in Figs. 14 and 15, when recovering AP amplitude is plotted as a percentage of unmasked (control) amplitude, the recovery curves appear roughly parallel, except that when recovery amplitude reaches 85–90 % of control amplitude, the curves begin to converge. Also, the recovery curve for the −5

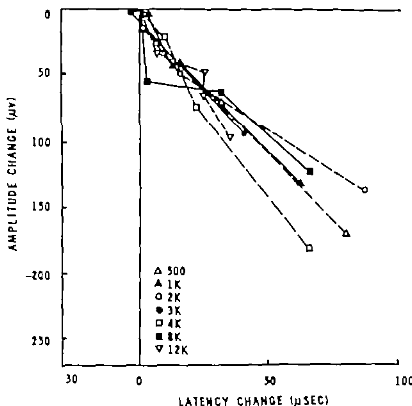


Fig 10 Latency change plotted against amplitude change at different masking frequencies. The data are the same as those plotted in Fig. 9

temperature at the long' masking duration seems to deviate slightly from the parallel course of the other curves

In studying the effects of varying test-click intensity we found that when test-click amplitude is varied the method of plotting recovery (i.e whether as a percentage of control or in absolute terms) has an effect on the apparent time course (Coats, 1967) Since changing temperature changes control amplitude, this effect also occurs with cooling Figs 16 and 17 show a replot of the recovery curves in Figs. 14 and 15 in terms of the difference between recovering and control amplitudes. These plots bear a striking resemblance to similar plots of recovery from masking at different test-click intensities (Coats, 1967) At relatively low control amplitudes (at low test-click intensities or at low temperatures) masking reduces AP amplitude less (in absolute terms) than at higher control amplitudes. As control amplitude increases (by increasing either temperature or click intensity) the difference between masked and unmasked AP amplitudes reaches a constant ("plateau") level. However regardless of control amplitude the time required for complete recovery remains constant. The experimental results thus lead to the conclusion that temperature affects masking in a way which resembles the effects of changing masking or test-click intensity rather than masking duration

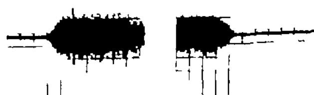


Fig 11 Condenser microphone record of onset and cutoff of the white-noise masking stimulus used to obtain the plots shown in Figs. 1 and 13 One horizontal division = 5 msec. Rise-fall time was 7 5 msec. Sound intensity was 50 dB, SPL. Each record is 10 superimposed oscilloscope traces.

The effects of masking cooling and attenuation on click-pair recovery

By measuring the amplitude of the second AP response at various interclick intervals, a click

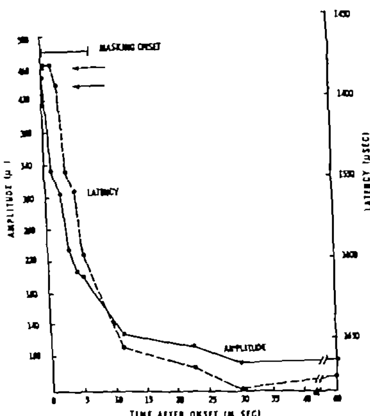


Fig 12 Time course of amplitude decline and latency increase at the onset of white-noise masking. The latency scale is inverted to facilitate comparison. Arrows at the left show unmasked values. Each data point was measured from an average of 25 round-window responses. Masking rise-fall time was 7.5 msec. Click intensity was 75 dB, peak-equivalent SPL.

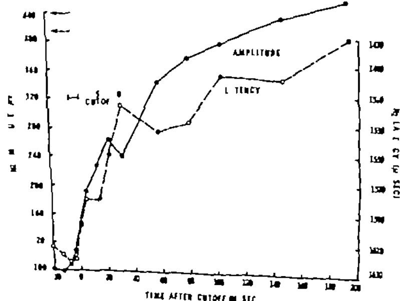


Fig 13 Time course of amplitude and latency recovery during and after cutoff of the same white-noise masking stimulus which produced the plots shown in Fig. 12 (the latency scale has been inverted). The time scale is compressed in comparison with the time scale in Fig. 12. The masking stimulus lasted 0.5 sec.

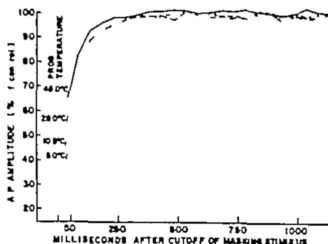


Fig 14 Click AP amplitude recovery following short duration (0 1. sec) masking at different temperatures. Temperature was controlled with a thermoelectric cold probe. The masking stimulus was a 65-dB, 2.5-kHz tone with a 7.5 msec rise–fall. Click intensity was 65 dB peak-equivalent SPL.

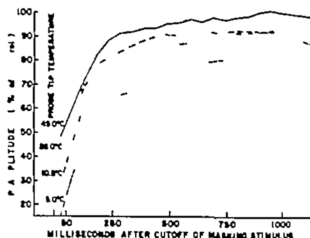
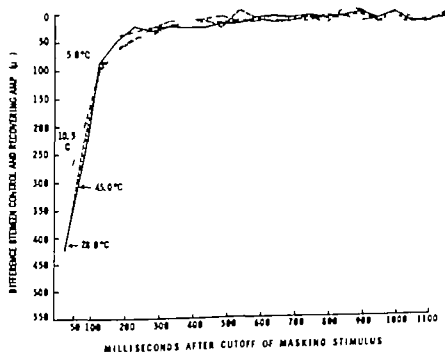


Fig 15 Click AP amplitude recovery following long duration (.4 sec) masking. All other parameters are the same as listed in the caption for Fig. 14.

pair recovery curve" can be plotted (Sørensen 1959 Dewson 1967) We have obtained additional information on the differential effects of masking, cooling, and attenuation from a series of experiments in which click pair recovery was compared under the three conditions

In each experiment, we obtained a control" click pair recovery curve using clicks of moderate intensity Masking, cooling, and attenuation reduced the control AP amplitude by about 50% with the parameters of each chosen to produce equal AP reduction Each of the three conditions yielded a click pair recovery curve which we compared with the control curve.

Fig. 18 illustrates typical results. Masked click pair recovery curves always reached con



F g 16 Click AP amplitude recovery at different temperatures following short-duration (0 12 sec) masking. These are the same curves as shown in Fig. 14 except that recovering AP amplitude is plotted in terms of difference between control (unmasked) and recovering amplitudes rather than as a percentage of control amplitude.

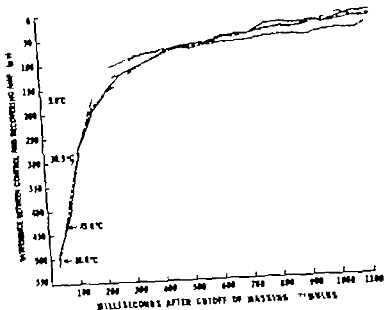


Fig. 17. Click AP amplitude recovery at different temperatures following long-duration (2.4 sec) masking. These are the same curves as shown in Fig. 15 except the recovering AP amplitude is plotted in terms of difference between control and recovering amplitudes rather than as a percentage of control amplitude.

trol values before control, attenuated, and cooled recovery curves. The attenuated and cooled recovery curves were always superimposable. The relationship between the attenuated and cooled recovery curves and the control recovery curve showed some variability. In three of eight experiments, the attenuated and cooled curves were superimposable on the control curves. In one experiment, attenuation and cooling seemed to slightly increase recovery rate. In the remaining four experiments, attenuation and cooling slowed recovery (see Fig. 18). In the four experiments in which cooling and attenuation failed to slow recovery, the intensity of the control click was at the lower end of the range used. It therefore seems possible that this variability is due to variation in control-click intensity.

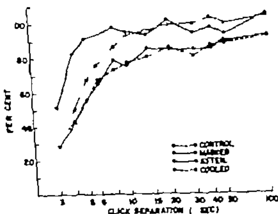


Fig. 18. Click-pair recovery curves with APs attenuated, cooled, and masked. The control-click intensity was 50 dB, peak-equivalent SPL, and the attenuated-click intensity was 35 dB, peak-equivalent SPL. We masked the clicks with white noise of 20 dB, SPL, and cooled with the probe-tip temperature at 10.0°C. The curves were obtained by measuring averages of 20 APs.

Discussion

In this attempt to interpret the above results, the following assumptions are implicit (1) The whole-nerve AP amplitude is determined by the number of fibers firing synchronously in response to the click. Alterations in AP amplitude are produced by changing the number of synchronously firing fibers (2) The AP peak latency is determined by the modal latency of the single fiber responses.

Effects of cooling and attenuation

In a previous report (Coats, 1965) we speculated that the quantitatively equal effects of cooling and attenuation on AP latency were due to the two treatments having a common mechanism of action We further suggested that cooling and attenuation slow the rise time (hence increasing AP latency) and reduce the amplitude (hence decreasing AP amplitude) of a hypothetical cochlear excitatory process. Since the change in microphonic amplitude caused by cooling and attenuation differed by an order of magnitude, this hypothetical excitatory process must be central to the microphonic generator

The present results establish that temperature and stimulus intensity have qualitatively similar effects on masking recovery and quantitatively equal effects on click pair recovery This enlarged list of similarities between temperature and stimulus intensity further supports the thesis that the two treatments act via a common mechanism.

The broadening of the *N* peak which occurs with cooling and attenuation is easily compatible with this hypothesis, since it could be attributed to the well known increased variability of single fiber response latency which occurs near threshold

Our data do not rule out the possibility that temperature affects the click AP by direct action on the nerve fibers rather than on the process which excites the nerve fibers. However if we assume a direct temperature effect on the auditory-nerve fibers, it becomes difficult to explain the similarities between cooling and attenuation, since attenuation would not be expected to change spike height or threshold of individual nerve fibers.

Effect of masking

The slight latency increase accompanying masking could be explained by assuming that a traveling wave, which begins at the basilar end of the cochlear partition and moves apically generates the click AP and by also assuming that the masking stimulus selectively removes the responses of basilar fibers. However this explanation is considered unlikely because the present investigation shows no differential masking-frequency effect on AP latency

It seems probable that in the present investigation we are dealing with a patch of sensory epithelium and its associated afferent nerve fibers which respond more or less equally to all stimulus frequencies (Tasaki & Fernández, 1952 Kiang et al. 1963) The results must therefore be explained on the basis of the neurophysiological properties of this system rather than its mechanical properties.

One way to explain the latency increase produced by masking is to assume that two mechanisms are operative—one the classical line busy mechanism the other depression of the hypothetical "presynaptic" cochlear excitatory process (Coats, 1964 *a*) The line-busy mechanism would not be expected to increase latency and could therefore be invoked to account for most of the AP reduction produced by masking. Depressing the presynaptic process could then be regarded as a secondary effect, which would account for the slight latency increase having the same qualitative properties as the latency increase produced by cooling and attenuation. Recovery of the excitatory process would also explain why recovery from masking is much slower than would be expected if only the line-busy mechanism were operative

Latency change at onset and after cutoff of masking is, however, not compatible with a "dual-mechanism" explanation of physiological masking. Implicit in the dual-mechanism hypothesis is the assumption that line-busy depression would recover much more rapidly than excitatory-process depression. Hence, we would expect that the early "rapid" recovery phase would be dominated by line-busy depression. Therefore, during the early phase, latency recovery should markedly lag amplitude recovery. In actuality we found no differential effects on latency during the early and late phases of masking recovery.

A third argument against this hypothesis is that it makes the increased rate of masked click-pair recovery difficult to explain. Since the second of a pair of click APs remains depressed about 50 msec, we would assume that the time course of click-pair recovery is almost solely accounted for by excitatory-process recovery. But, according to the dual-mechanism hypothesis, the masking stimulus should remove units almost entirely by the (postsynaptic) line-busy mechanism, and this would not be expected to alter the recovery of the excitatory process.

A third possible explanation of the experimental results is that physiological masking results from a line-busy effect, but that the post-stimulus depression, which causes the effect, recovers much more slowly than the refractory period of myelinated nerve fibers. This hypothesis leads to a straightforward explanation of all the results thus far obtained. We must, however, make one qualification. Since repetitive firing of single auditory-nerve fibers occurs at rates of up to 1 000/sec (Tasaki, 1954; Kiang et al., 1963; Rose et al., 1967), the slowly recovering, post-stimulus depression must be conceived of as having an effect on the probability of firing throughout the responding population rather than as having an invariant effect on each fiber.

It is well known that recovery processes in unmyelinated nerve fibers are much slower than in myelinated fibers, and it is therefore tempting to place the hypothetical, slowly recovering process in the unmyelinated auditory-nerve endings, which extend from the habenula perforata to the terminations on the hair cells. Since post-stimulus depression in unmyelinated nerve fibers is cumulative, even with relatively long interstimulus intervals, this hypothesis can account for the masking-duration effect.

Previous experiments established that shortening masking duration increases recovery rate (Coats, 1964 a). At very short masking durations (less than 50 msec), the time required for recovery approaches a limit of about 50–100 msec. If we assume that only a single process (a slowly recovering poststimulus depression of excitability) accounts for masking, then the click-pair recovery situation can be regarded as equivalent to shortening the masking stimulus to the limit. The increased rate of click-pair recovery produced by masking is then easily explained, since it is logical to assume that those fibers which still respond to the click in the presence of the masking stimulus are (or tend to be) more rapidly recovering.

The observed effects of masking on latency can also be explained in a straightforward way. At high click intensities, where relative increase in latency with masking is greatest, the masking stimulus would be expected to eliminate from the population responding to the click only those fibers with relatively low thresholds. The presynaptic excitatory process would then take longer to reach the thresholds of the remaining fibers, thus causing a latency increase with a qualitative similarity to the latency increase produced by cooling and attenuation. However, the latency increase would be determined by the distribution of thresholds among the population of responding fibers rather than the rise time of the excitatory process—hence, the observed quantitative difference.

At intermediate test-click intensities, latency would be expected to increase as masking intensity increased from low levels. But as masking intensity reaches and surpasses the highest thresholds of the fibers responding to the click,

Discussion

In this attempt to interpret the above results, the following assumptions are implicit. (1) The whole-nerve AP amplitude is determined by the number of fibers firing synchronously in response to the click. Alterations in AP amplitude are produced by changing the number of synchronously firing fibers. (2) The AP peak latency is determined by the modal latency of the single-fiber responses

Effects of cooling and attenuation

In a previous report (Coats 1965) we speculated that the quantitatively equal effects of cooling and attenuation on AP latency were due to the two treatments having a common mechnism of action We further suggested that cooling and attenuation slow the rise time (hence increasing AP latency) and reduce the amplitude (hence decreasing AP amplitude) of a hypothetical cochlear excitatory process. Since the change in microphonic amplitude caused by cooling and attenuation differed by an order of magnitude this hypothetical excitatory process must be central to the microphonic generator

The present results establish that temperature and stimulus intensity have qualitatively similar effects on masking recovery and quantitatively equal effects on click pair recovery This enlarged list of similarities between temperature and stimulus intensity further supports the thesis that the two treatments act via a common mechanism.

The broadening of the N_1 peak which occurs with cooling and attenuation is easily compatible with this hypothesis since it could be attributed to the well known increased variability of single-fiber response latency which occurs near threshold

Our data do not rule out the possibility that temperature affects the click AP by direct action on the nerve fibers rather than on the process which excites the nerve fibers. However if we assume a direct temperature effect on the auditory nerve fibers, it becomes difficult to explain the similarities between cooling and attenuation since attenuation would not be expected to change spike height or threshold of individual nerve fibers.

Effect of masking

The slight latency increase accompanying masking could be explained by assuming that a traveling wave, which begins at the basilar end of the cochlear partition and moves apically generates the click AP and by also assuming that the masking stimulus selectively removes the responses of basilar fibers. However this explanation is considered unlikely because the present investigation shows no differential masking-frequency effect on AP latency

It seems probable that in the present investigation we are dealing with a patch of sensory epithelium and its associated afferent nerve fibers which respond more or less equally to all stimulus frequencies (Tasaki & Fernández, 1952 Kiang et al. 1963) The results must therefore be explained on the basis of the neurophysiological properties of this system rather than its mechanical properties.

One way to explain the latency increase produced by masking is to assume that two mechanisms are operative—one, the classical line-busy mechanism the other depression of the hypothetical presynaptic" cochlear excitatory process (Coats, 1964 *a*) The line-busy mechanism would not be expected to increase latency and could therefore be invoked to account for most of the AP reduction produced by masking. Depressing the presynaptic process could then be regarded as a secondary effect which would account for the slight latency increase having the same qualitative properties as the latency increase produced by cooling and attenuation Recovery of the excitatory process would also explain why recovery from masking is much slower than would be expected if only the line busy mechanism were operative

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the probability of a given fiber's being masked would be determined less and less by threshold, and latency would therefore decline again toward control values.

The relatively small latency change at low click intensities is also easily accounted for since we would expect the threshold range of fibers responding to the low intensity click to be relatively small.

Summary

We compared the effects of cooling, stimulus attenuation and physiological masking on the gross click action-potential (AP) and found that. (1) cooling and attenuation produce equal increases in AP latency (2) cooling and attenuation have equal effects on click pair recovery and on masking recovery (3) masking increases AP latency but by a much smaller amount than cooling and attenuation, and the effect is dependent on masking and test-click intensity (4) the frequency of the masking stimulus has no effect on the amount of increase in masked AP latency (5) during onset of and recovery from masking, AP latency and AP amplitude appear time locked. The results are most easily explained if we assume that: (1) cooling and attenuation act on a postmicrophonic cochlear excitatory process, (2) the mechanism of physiological masking is a line-busy effect, but the poststimulus depression of excitability recovers much more slowly than the refractory period of myelinated nerve fibers.

Zusammenfassung

Wir haben die Wirkungen von Kühlung, Reizabschwächung und physiologischer Maskierung auf den gesamten Klick Aktionspotentialen (AP) miteinander verglichen und dabei herausgefunden, dass (1) Kühlung und Abschwächung gleiche Zunahme von AP Latenz hervorrufen (2) Kühlung und Abschwächung gleiche Wirkungen auf Klick Paar Wiederherstellung und Maskierungs-Wiederherstellung haben (3) Maskierung die AP Latenz verstärkt jedoch in sehr viel geringerem Masse als Kühlung und Abschwächung, und die Wirkung hängt von der Maskierung und von der Test Klick Intensität ab (4) die Frequenz des Maskierungsreizes keine Wirkung auf das Mass der Zunahme bei maskierter AP Latenz hat (5) während des Anfalls und der Genesung von Maskierung, AP Latenz und AP Umfang zeitgebunden erscheinen. Die Ergebnisse sind höchst einfach zu erklären, wenn wir annehmen dass. (1) Kühlung und Abschwächung in einem postmikrophonischen Reizungsprozess vorsichgehen, (2) der Mechanismus der physiologischen Maskierung ein dauertönender Effekt ist, aber dass die nachwirkende Schwäche der Erregbarkeit sich sehr viel langsamer wieder erholt als die langanhaltende Periode myelinierter Nervenfasern.

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CONTENTS

I Introduction

The mouse provides excellent material for the study of the normal and abnormal development of the mammalian inner ear. It lends itself to the study of hereditary malformations of the inner ear because there are twenty-five known genes affecting the inner ear of the mouse and some of these lead to lesions which resemble lesions occurring in man.

Various investigators have studied aspects of the normal development of the inner ear of the mouse in order to evaluate deviations from the normal pattern of development. M. S. Deol (1, 2, 3, 4), Kikuchi and Hilding (9), Gruneberg, Hallpike and Ledoux (5) and Truslove (19) have studied the differentiation of a number of mutant strains. Each of these authors has described some features of the differentiation of the inner ear of the normal mouse. Ingalls, Kelemen, and Curley (7) in discussing the effect of hypoxia on the development of the inner ears of embryos of pregnant mice, discuss features of the inner ears of the neonates.

Mikaelian and Ruben (12) have studied the correlation of the appearance of the cochlear potential, nerve action potentials, and behavioral responses with the cochlear anatomy in the developing mouse. They have described some aspects of the postnatal anatomical development of the inner ear. Otis and Brent (14) in comparing the rate of development of the human embryo with that of the mouse embryo, have timed the appearance of some of the major features of the otic labyrinth in the mouse.

Weibel (20) used the light microscope to make a detailed morphological study of the inner ear of the mouse after birth. He did not deal with the details of the differentiation processes taking place before birth. Kikuchi and Hilding (8) used phase contrast and electron microscopy to study the maturation of the organ of Corti in mice from birth to adulthood. They also studied the development of the stria vascularis in mice after birth (10). Ruben (17) has studied the developmental pattern of terminal mitoses in the membranous labyrinth. Ramon y Cajal (15) and Tello (18) have studied the pattern of innervation of the developing inner ear of the mouse.

None of the above studies provides a comprehensive histological picture of the embryological and postnatal development of the inner ear of the mouse. This report provides a day by day analysis of the anatomical and histological changes taking place in the inner ear of the normal mouse from the eleventh day of gestation to the tenth day after birth. The development of the patterns of innervation of the vestibular apparatus and cochlear duct is discussed. It is hoped that this paper will facilitate comparative studies of the embryonic and postnatal development of the malformed inner ears of the many genetically deaf strains with that of the normal organ. It also will provide a model of *in vivo* development of the normal inner ear by which the progress of mouse otocysts grown *in vitro* can be measured.

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II Procedure

Tissue processing

A series of twenty two embryos and twenty young mice of the CBA J/CBA J strain was studied Either two or four embryos for each day of gestation from the eleventh to the nine teenth were examined. Similarly two mice were sacrificed on each day after birth from the first to the tenth.

Ears from two different litters were ex amined from the eleventh day of gestation through the sixteenth day of gestation Ears from members of only one litter per day were used for the observations on the remainder of the days Only one ear of each specimen was analyzed Right or left ears were used on a random basis No significant differences were found between ears of litter mates or those of non-litter mates of the same age In one case only a difference in development equivalent to about one half day of gestation was noted between two non litter mates of the same age.

Dating of the embryos was accomplished by means of the vaginal plug method. Males and females were put together in one cage over night and the females were checked for vagi nal plugs at 8 00 a m the following day The day when the plug was observed was called the first day of gestation The day of birth was called the first day postpartum All of the postpartum animals studied were born on the twentieth day of gestation.

The pregnant females were sacrificed on the appropriate day by cervical dislocation The abdomen was opened and the embryos were removed from the uterus and placed in a 0 9% solution of sodium chloride in water Embryos older than sixteen days were decapitated and only their heads were preserved The em bryos were fixed in 10% Acrolein solution buffered with phosphate to pH 7 4 Embryos younger than sixteen days were kept in the Acrolein solution for three hours. The heads of embryos seventeen days or older were placed in Acrolein overnight at four degrees Centigrade in the refrigerator

The postpartum mice were decapitated. The skin and mandible were removed from the head. The nose was cut off anterior to the eyes and the head was divided in the sagittal plane. Each part was then placed in the Acrolein solution for twenty four hours.

After fixation specimens were washed in tap water for one-half hour Specimens younger than fifteen days of gestation were then de hydrated in a 1 1 (v/v) solution of ethylene glycol monomethyl ether and methyl alcohol for forty eight hours. This solution will be referred to as M & M Specimens older than fifteen days of gestation were decalcified before being dehydrated This was accomplished over a three-day period by a 10% solution of di-sodium ethylenediamine tetra acetate (EDTA) with 2 5% of polyvinylpyrrolidone buffered to pH 7 2 with a Tris hydroxymethyl amino-methane buffer The specimens were then washed in tap water for one half hour and dehydrated in M & M for three to five days.

Those specimens which had been decal cified in EDTA were after being dehydrated in M & M placed in 2% celloidin dissolved in 1 1 ethylene glycol monomethyl ether and methyl alcohol for twenty four hours This was followed by hardening in chloroform for twelve hours This last procedure was not carried out with those specimens which had not been decalcified

All specimens were placed in 90 poly ethylene glycol 400-distearate and 10 cetyl alcohol (w/w) at thirty seven degrees Centi grade for 72 hours. The tissue was embedded in the polyethylene glycol 400-distearate and cetyl alcohol and was placed in the refrigerator until it was sectioned

Embryos of thirteen days of gestation or

less were sectioned at 5 microns. Those of *fourteen* to seventeen days of gestation were sectioned at 7 microns. All specimens older than seventeen days of gestation were sectioned at 10 microns. All sections were serially mounted.

At least one specimen at each age was stained with Harris hematoxylin and eosin Y. One specimen at each level of development was stained by the Protargol technique of Rasmussen and McCrane (16).

Methods of observation

The degree of coiling of the cochlear duct was determined for each specimen by an adaptation of the graphic reconstruction method of Guild (6). This method projects the turns of the cochlear duct onto a common plane perpendicular to the axis of the cochlear duct. The method described by Guild uses as its baseline the center of the unions of the outer and inner pillar cells. In the early embryological inner ear the cochlear duct has not undergone sufficient cytological differentiation to allow identification of inner and outer pillar cells. Therefore the tangent to the outermost cells of the undifferentiated axial wall of the cochlear duct was used to reconstruct these early cochlear ducts.

III Development of the Otic Labyrinth

A Eleventh and Twelfth Day Otocysts

The eleventh day otocyst is a closed ovoid sac (Plate 1 Fig. A). The dorsal part of the caudo-lateral wall consists of simple cuboidal epithelium, and the dorsal part of the medial wall is made up of pseudostratified columnar epithelium *with two to three layers of nuclei*. The cells of the caudal and rostral walls are pseudostratified columnar and merge with the simple low cuboidal cells of the caudo-lateral wall. The ventral part of the otocyst consists entirely of pseudostratified epithelium with about three layers of nuclei.

At the twelfth day of gestation the endolymphatic duct has developed as an evagination from the medial wall of the otocyst about one-third of the way from the dorsal to the ventral ends (*Plate 1 Fig. B*). *The walls of* the endolymphatic duct consist of simple cuboidal *epithelium*. The medial and lateral walls of the dorsal part of the twelve day otocyst are made up of simple cuboidal epithelium. The rostral and caudal surfaces of the dorsal part are pseudostratified columnar epithelium (Plate 1 Fig. C). Ventral to the opening of the endolymphatic duct the walls of the otocyst are pseudostratified columnar epithelium (Plate 1 *Fig. D*). Thus, while the dorsal portion of the otocyst consists largely of simple epithelium, *the nuclei of the ventral part are stratified in two to three layers*. The ganglion of the eighth nerve lies medial and somewhat rostral to the thicker ventral part of the otocyst (Plate 1 Fig. D). A ridge of pseudostratified columnar epithelium is present on the lateral wall, directly opposite the point where the endolymphatic duct meets the otocyst (Plate 1 Fig. B). The ridge is where the utriculoendolymphatic valve *and utriculosaccular duct* will form.

B Development of the Semicircular Ducts

The superior semicircular duct is the first semicircular duct to develop. It appears *in its most* rudimentary *form* between the twelfth and thirteenth *days of gestation* as a pouch of epi-

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together at the opening of the saccule. It does not extend across the opening of the utricle. The valve is considerably longer on the seventeenth day of gestation, extending laterally across the opening of the utricle (Plate 4 Fig. B). It changes the configuration of the utriculosaccular connection from a straight line to a right angle bend by covering the mouth of the utricle. It also causes the endolymphatic duct to flow directly into the saccule. The valve is made up of the low cuboidal epithelium of the utricular wall, the slightly taller cuboidal epithelium of the endolymphatic duct, and the periotic tissue of the vestibule between the two epithelial layers (Plate 4 Fig. C) The utriculoendolymphatic valve has grown into a crescent shaped flap at nineteen days and completely covers the opening of the utricle. The concave side of the flap faces inside the utricle (Plate 4 Figs. D and E).

The maculae of the utricle and saccule start to differentiate between the thirteenth and fourteenth days of gestation. In the thirteen day embryo there is a small portion of the wall of the otocyst which has five to six layers of densely packed nuclei topped by a layer of eosinophilic cytoplasm. This region is located at the point where the rostral loop of the superior semicircular duct opens into the utricle (Plate 5 Figs. A and B) It may be the forerunner of sensory epithelium of the crista of the superior semicircular duct or of the macula of the utricle. The maculae of both the utricle and saccule have clearly differentiated at the fourteenth day of gestation (Plate 5 Fig. C). The macula of the utricle is present on the lateral and rostral walls of the utricle in the vicinity of the ampulla of the lateral semicircular duct. The macula of the saccule is located on the medial wall of the saccule. On the fourteenth day of gestation both maculae have a stratum of supporting cells which contains five to six rows of densely packed nuclei and is topped by a single row of tall columnar cells. The latter appear to be the precursors of the sensory cells. They are much larger than the supporting cells and have a great deal of very clear eosinophilic cytoplasm. The cytoplasm of the supporting cells is not seen and the nuclei are closely packed. The nuclei of the sensory cells are much larger than the nuclei of the supporting cells. The nuclei of the supporting cells have heavy chromatin markings, whereas the nuclei of the sensory cells are almost glassy clear with few dark areas. Any chromatin markings that are present are much fainter and smaller than those in the supporting cells. On the sixteenth day there appears to be less interdigitation between sensory and supporting cells than on the preceding days of development (Plate 6 Fig. A) In some regions there is a space between the top layer of cells and those below On the seventeenth day this gap is still apparent (Plate 6 Fig. B). It may be artifactual, or it may represent an anatomic separation between the sensory and supporting cells.

Between the fourteenth and seventeenth days the nuclei of the tall columnar cells become progressively larger and more glassy in appearance. From the sixteenth to the eighteenth days of gestation the number of rows of supporting cell nuclei in the maculae decreases to two or three (Plate 6 Fig. C) Only one layer of supporting cell nuclei is present on the nineteenth day (Plate 6, Fig. D) The result is stratified epithelium of two layers. On the nineteenth day the sensory cell nuclei are large, round and much lighter staining than the nuclei of the supporting cells. The nuclei of the supporting cells are ovoid and have heavier and more numerous chromatin spots than the sensory cell nuclei. Almost no cytoplasm is visible around the supporting cell nuclei, but the sensory cells have a great deal of cytoplasm and are about three times as tall as they are wide. Otoconia are present for the first time on the seventeenth day The number of otoconia increases considerably on the eighteenth and nineteenth days. On the day of birth both maculae have thick otolithic membranes with large numbers of otoconia.

The non-maculated walls of the utricle and saccule consist of simple cuboidal epithelium.

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The cristae are papillae which on the fourteenth day of gestation have a stratum of supporting cells with five to six levels of closely packed nuclei This stratum is topped by a single layer of tall columnar cells (Plate 3 Fig A) There is little cytoplasm in the supporting cells The supporting cell nuclei are ovoid and have numerous darkly staining basophilic granules The cells in the top row have a great deal of clear eosinophilic cytoplasm and large, clear basal nuclei They may be the forerunners of the sensory epithelium of the cristae. Their nuclei are larger and paler staining than the supporting cell nuclei below On the seventeenth day of gestation the periotic connective tissue and fibers of the eighth nerve are present in the papilla of supporting cells so that the cristae have cores of connective tissue (Plate 3 Fig. B) The number of strata of supporting cell nuclei has decreased to three or four At the nineteenth day of development the number of layers of supporting cell nuclei has decreased to less than three from the original five to six layers (Plate 3 Fig. C). In some regions there is only one layer of supporting cell nuclei On the first day after birth the cristae have one layer of supporting cells topped by a layer of sensory cells (Plate 3 Fig. D) The sensory cells are tall columnar cells about three times as tall as they are wide. Their nuclei are round and lighter staining than the ovoid nuclei of the supporting cells. The chromatin of the sensory cell nuclei is much finer and less clumped than that of the supporting cell nuclei. The cristae are well differentiated by the first day after birth.

C Development of the Utricle Saccule and Utriculoendolymphatic Valve

The first step toward separation of the membranous labyrinth into utricle and saccule occurs on the fifteenth day of gestation A constriction appears in the region of the origin of the endolymphatic duct and represents the beginning of formation of the utriculosaccular duct (Plate 4 Fig. A) The newly separated utricle and saccule lie along a straight line, and the utriculosaccular duct at this stage of development is a straight channel The endolymphatic duct lies medial to the utricle and opens into the short utriculosaccular duct.

The utriculoendolymphatic valve is present in rudimentary form on the fifteenth day of gestation. It is represented by a small fold of tissue consisting of the reflection of the epithelial walls of the utricle and endolymphatic duct at the point where the two structures flow

together at the opening of the saccule. It does not extend across the opening of the utricle. The valve is considerably longer on the seventeenth day of gestation, extending laterally across the opening of the utricle (Plate 4 Fig. B). It changes the configuration of the utriculosaccular connection from a straight line to a right angle bend by covering the mouth of the utricle. It also causes the endolymphatic duct to flow directly into the saccule. The valve is made up of the low cuboidal epithelium of the utricular wall, the slightly taller cuboidal epithelium of the endolymphatic duct, and the periotic tissue of the vestibule between the two epithelial layers (Plate 4 Fig. C). The utriculoendolymphatic valve has grown into a crescent shaped flap at nineteen days and completely covers the opening of the utricle. The concave side of the flap faces inside the utricle (Plate 4 Figs D and E).

The maculae of the utricle and saccule start to differentiate between the thirteenth and fourteenth days of gestation. In the thirteen day embryo there is a small portion of the wall of the otocyst which has five to six layers of densely packed nuclei topped by a layer of eosinophilic cytoplasm. This region is located at the point where the rostral loop of the superior semicircular duct opens into the utricle (Plate 5 Figs. A and B). It may be the forerunner of sensory epithelium of the crista of the superior semicircular duct or of the macula of the utricle. The maculae of both the utricle and saccule have clearly differentiated at the fourteenth day of gestation (Plate 5 Fig. C). The macula of the utricle is present on the lateral and rostral walls of the utricle in the vicinity of the ampulla of the lateral semicircular duct. The macula of the saccule is located on the medial wall of the saccule. On the fourteenth day of gestation both maculae have a stratum of supporting cells which contains five to six rows of densely packed nuclei and is topped by a single row of tall columnar cells. The latter appear to be the precursors of the sensory cells. They are much larger than the supporting cells and have a great deal of very clear eosinophilic cytoplasm. The cytoplasm of the supporting cells is not seen and the nuclei are closely packed. The nuclei of the sensory cells are much larger than the nuclei of the supporting cells. The nuclei of the supporting cells have heavy chromatin markings, whereas the nuclei of the sensory cells are almost glassy clear with few dark areas. Any chromatin markings that are present are much fainter and smaller than those in the supporting cells. On the sixteenth day there appears to be less interdigitation between sensory and supporting cells than on the preceding days of development (Plate 6 Fig. A). In some regions there is a space between the top layer of cells and those below. On the seventeenth day this gap is still apparent (Plate 6, Fig. B). It may be artifactual, or it may represent an anatomic separation between the sensory and supporting cells.

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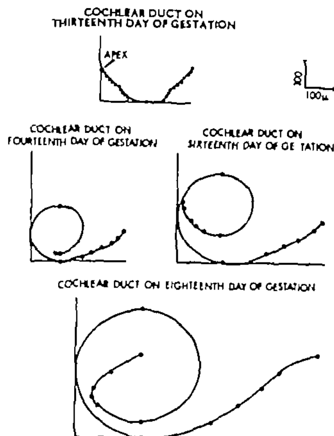
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Graph 1 Development of the cochlear duct The reconstructions demonstrate the increase in size and *degree of coiling* of the cochlear duct from the thirteenth to the eighteenth days of gestation

D Development of the Cochlear Duct

1 Formation of cochlear duct and ductus reuniens

The cochlear duct starts to develop between the twelfth and thirteenth days of gestation as an extension of the ventral part of the otocyst The thirteen day embryo has about one-*half turn* of cochlear duct The duct has the form of a half coil with a hook near the distal end (Graph 1) There is no ductus reuniens, and a wide channel connects the cochlear duct with the vestibular labyrinth The cochlear duct on the fourteenth day is about one and one-quarter coils long (Graph 1) On the fifteenth day the wide channel between the cochlear duct and the saccule begins to narrow This forerunner of the ductus reuniens consists of pseudostratified epithelium with two layers of nuclei on its lateral side and more than five layers on its medial side (Plate 7 Fig. A) The thicker side is continuous *dorsally* with the macula of *the saccule* and ventrally with the thick caudal wall of the cochlear duct.

On the sixteenth day of gestation the cochlear duct has about one and one-half coils (Graph 1) The ductus reuniens is well defined. Its lateral wall consists of simple cuboidal epithelium and its medial wall consists of pseudostratified cuboidal epithelium (Plate 7 Fig. B) Between the seventeenth and eighteenth days of gestation the cochlear duct has between one and one half and one and three-quarters coils There is no further coiling of the cochlear duct (Graph 1) On the eighteenth day of gestation all of the walls of the ductus reuniens consist of simple cuboidal epithelium (Plate 7 Fig. C)

2. Differentiation of the organ of Corti

The cochlear duct on the thirteenth day has two types of walls (Plate 8 Fig. A) The rostral wall consists of epithelium with two to three layers of tightly packed nuclei The caudal wall has six layers of tightly packed nuclei

The caudal wall of the cochlear duct is the site of differentiation of the organ of Corti. On the thirteenth day of development differentiation into sensory and supporting cells has not taken place On the fourteenth day the caudal wall has differentiated into a layer of supporting cells containing five to six strata of nuclei The supporting cell layer is topped by a layer of tall columnar cells which have basal nuclei and clear eosinophilic cytoplasm (Plate 8 Fig. B) It resembles the maculae of the utricle and saccule on the same day of development.

The appearance of the caudal wall does not change significantly on the fifteenth and sixteenth days of gestation (Plate 8 Figs. C D E) The sensory cells in the cochlear duct have more cytoplasm than the supporting cells. However unlike the sensory cell nuclei of the maculae of the utricle and saccule, the nuclei of the sensory cells of the cochlear duct are not markedly different from the nuclei of the supporting cells

On the seventeenth day the sensory cells in the lateral portion of the basal third of the duct show signs of differentiation into three outer hair cells, one inner hair cell, and phalangeal cells (Plate 9 Figs. A, B). The hair cells rest on a layer of supporting cells that has one to two strata of nuclei. The nuclei of the hair cells stand somewhat higher than the nuclei of adjacent cells. Between the outer hair cells and the inner hair cell is an area of highly vacuolated cytoplasm. The caudal wall of the apical two-thirds of the cochlear duct has not progressed in differentiation from the sixteenth day (Plate 9 Figs. C D).

The area which will form the tunnel of Corti has become apparent in the basal and middle coils on the eighteenth day of development (Plate 10 Figs. A, B C). The cytoplasm is vacuolated and gives the appearance of a space. However there is still a cell in the space as cytoplasm and nuclei can be seen. Three outer and one inner hair cells flank the tunnel. They rest on a layer of supporting cells which, in the basal coil, has a single layer of nuclei. In the middle portion of the cochlear duct there are two to three layers of supporting cell nuclei beneath the hair cells. The differentiation of the apical coil on the eighteenth day is not significantly greater than on the seventeenth day (Plate 10 Fig. D)

The outer and inner hair cells have differentiated throughout the length of the cochlear duct on the nineteenth day of gestation (Plate 11 Fig. A) At nineteen days of gestation the tunnel of Corti is forming. The inner and outer hair cells are tilted over the tunnel in such a way that their apices lean toward one another The region of the tunnel is not a free space but consists of vacuolated cytoplasm. There are nuclei in the space where the tunnel is forming. The pillar shaped cytoplasmic processes of the inner and outer pillar cells are not differentiated. The pillar cells gradually become thinner after the birth of the animal.

The tectorial membrane begins to form by the first day postpartum. It is represented at that time by a thin strip of pale eosinophilic material over the cells in the region where the sulcus spiralis internus will form (Plate 11 Fig B). This is the first day on which a definitive structure representing the tectorial membrane is visible. On previous days small areas of pale eosinophilic material could occasionally be identified in the region where the tectorial membrane later develops. These may represent earlier stages of tectorial membrane formation. Different staining techniques would help to determine on which day of development formation of the tectorial membrane commences. The tectorial membrane thickens on subsequent days of development (Plate 12 Figs. A B, C)

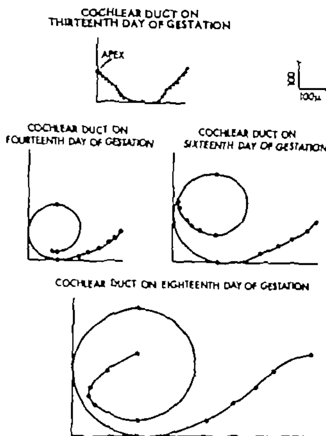
Between the fifth and sixth days postpartum the nuclei of the inner and outer pillar cells move to opposite sides of the tunnel of Corti (Plate 12, Figs. A, B). The pillar cells attain their mature shape on the tenth day post partum. The tunnel of Corti becomes wider between the eighth and tenth days after birth (Plate 12, Figs. C, D)

Between the eighth and tenth days post partum the hair cells become narrower This narrowing process leaves large gaps between the outer hair cells. The Deiters cells and the inner phalangeal cells become very vacuolated on the eighth and ninth days postpartum. On the tenth day these cells are very slender and there are large spaces between them. Thus, the space of Nuel is well differentiated by the tenth day postpartum (Plate 12, Figs. C D).

3 Differentiation of the limbus spiralis

The limbus spiralis starts to differentiate between the seventeenth and eighteenth days of gestation. In the eighteen day embryo, development of the limbus is limited to the basal coil of the cochlear duct, where the axial portion of the caudal wall has become simple cuboidal epithelium (Plate 10 Figs. A B C, D). Beneath this epithelial layer is highly cellular condensed mesenchyme.

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starting to differentiate into limbus, and the epithelial layer in the region of differentiation has about two strata of nuclei (Plate 13 Fig. A) In the basal coil the mesenchyme under the epithelium has become less cellular than it was at eighteen days and there is more extracellular matrix.

On the first day after birth the limbus is differentiated throughout the cochlear duct except for the extreme apex (Plate 13 Figs B C D) The limbus has started to develop in the apical coil but its epithelial wall has two to three strata of nuclei The connective tissue under the limbus in the basal and middle coils has a great deal of extracellular matrix. The region immediately adjacent to the epithelium consists almost entirely of extracellular matrix

On the fourth day after birth the limbus spiralis has formed throughout the cochlear duct. It consists of a mound of connective tissue covered by simple tall cuboidal epithelium with a single stratum of long thin nuclei Beneath the epithelium there is a wide band of connective tissue containing few cells The cell density of the connective tissue decreases in later development

4 Differentiation of the sulcus spiralis internus

The sulcus spiralis internus starts to develop after the first day postpartum On the first day postpartum the region just lateral to the limbus is still occupied by four to five layers of nuclei and a surface layer of eosinophilic cytoplasm (Plate 13 Figs. B C D) The epithelium is similar to that of the undifferentiated wall of the cochlear duct at sixteen days of gestation. On the second day after birth the cytoplasm of these cells is slightly vacuolated (Plate 14 Fig. A)

The number of layers of nuclei in the basal coil in the region of the sulcus has decreased to two or three by the sixth day after birth (Plate 14 Fig. B) Large amounts of cytoplasm in the sulcus region appear to have disintegrated The middle coil also has only two to three layers of nuclei in the region of the sulcus. The cytoplasm is degenerating and is filled with large vacuoles. Cytoplasmic disintegration is less complete than in the basal coil The apical coil shows little differentiation. On the seventh day the apical coil shows considerable vacuolization (Plate 14 Fig. C)

By the tenth day postpartum the sulcus spiralis internus has formed throughout the cochlear duct. It is lined by a layer of simple low cuboidal epithelium (Plate 14 Fig. D)

5 Differentiation of Reissner's membrane

Reissner's membrane develops from the rostral wall of the cochlear duct. The rostral wall of the cochlear duct of the thirteen day embryo consists of epithelium with two to three layers of tightly packed nuclei (Plate 15 Fig. A) On the fourteenth day of gestation the rostral wall still has two to three layers of nuclei but the extreme medial and lateral portions of the wall consist of simple columnar epithelium (Plate 15 Fig. B) The rostral wall of the fifteen day cochlear duct shows large areas particularly in the apical coil which have become simple cuboidal epithelium On the sixteenth day the rostral wall is composed of simple cuboidal epithelium throughout the duct (Plate 15 Figs. C D) This represents the inner cell layer of Reissner's membrane

On the nineteenth day of gestation, parts of the rostral wall of the basal turn have two layers of cells (Plate 16 Fig. A) The layer derived from the otocyst is simple cuboidal epithelium. Closely applied to this, on the outer surface is a single layer of long, thin cells The latter are derived from the mesenchyme surrounding the otocyst and remain when the scala vestibuli forms The other areas of the basal and middle coils have two to three layers of mesenchyme adjacent to the epithelial wall The apical coil has a thick condensation of mesenchyme adjacent to the epithelial wall since the scala vestibuli has not yet formed in the apical turn (Plate 16 Fig B) By the third day after birth the rostral wall of the entire basal and middle coils have been reduced to two layers of cells with only occasional adherence of additional mesenchymal cells to the

periotic side of the membrane. Reissner's membrane has differentiated throughout all turns of the cochlear duct by the fourth day after birth (Plate 16, Fig. C). On subsequent days of development, it appears stretched and somewhat thinner.

6. Differentiation of the stria vascularis

The stria vascularis starts to differentiate on the seventeenth day of gestation (Plate 17 Fig. A). A condensation of mesenchyme develops adjacent to the outer part of the rostral wall of the cochlear duct. This condensation contains many capillaries. On the nineteenth day the condensation is denser (Plate 17 Fig. B). The wall of the cochlear duct in this region consists of simple cuboidal epithelium. The cuboidal epithelial cells in the part of the wall adjacent to the condensed mesenchyme have nuclei that are slightly smaller and stain more intensely than those of the cells in the adjacent parts of the epithelial wall which are not developing into stria.

On the first day after birth the boundary between the epithelial wall and the underlying connective tissue is less distinct (Plate 17 Fig. C). The epithelial cells have become smaller and their nuclei are intensely stained with hematoxylin. The nuclei, which had been ovoid to round, are now elongated with the long axis of the nuclei at right angles to the plane of the connective tissue surface. The cells appear dentate, with projections going into the mesenchyme.

On the second day postpartum no sharp boundary is discernible between the epithelial wall and the condensed connective tissue (Plate 17 Fig. D). Between the second and third days after birth the mesenchyme becomes more compact. The cells are more tightly packed and their nuclei are smaller and more darkly staining.

On the fourth day there are two limiting walls of the stria (Plate 17 Fig. E). The inner one is derived from the otocyst. The outer one is from the mesenchyme. In between are capillaries intermingled with connective tissue cells. In subsequent development, the connective tissue of the stria becomes less cellular. It is still more cellular than the adult stria at ten days after birth.

IV Development of the Scala Vestibuli and Scala Tympani

On the twelfth day of gestation the mesenchyme around the otocyst is somewhat condensed. It is relatively sparse adjacent to the dorsal half of the otocyst and more dense adjacent to the ventral portion. At thirteen days the mesenchyme surrounding the otocyst is more condensed than at twelve days. The cochlear duct is completely surrounded by a layer of dense mesenchyme. On the fourteenth day of embryonic development the mesenchyme surrounding the cochlear duct shows differentiation into two concentric rings (Plate 18 Fig. A). The inner ring is less dense than the outer ring. Its interstitial substance stains more lightly and the nuclei are smaller. The nuclei in the outer, more darkly staining ring are larger and rounder than the ovoid nuclei of the inner zone. On the fifteenth day the outermost ring has considerable extracellular matrix and is starting to resemble cartilagenous tissue (Plate 18, Fig. B). Inside this newly forming cartilage is a region of loose connective tissue which shows regions of decreased density. No scala tympani or scala vestibuli have formed. On the sixteenth day there is still no differentiation of scalae vestibuli and tympani. The connective tissue in the inner ring is very sparse and the cytoplasm of the cells of this region form very fine trabeculae. The nuclei are small and stain darkly. The nuclei of the

surrounding ring are much larger and some what lighter staining There is a dense region of connective tissue cells lining the inner aspect of the cartilage ring

On the eighteenth day of gestation the scalae tympani and vestibuli have formed around the basal coil (Plate 18 *Fig.* C) They are represented by open cavities in the mass of connective tissue and are crossed by oc casional fine trabeculae Two to three layers of mesenchyme are condensed against the ves tibular side of Reissner's membrane. In the middle portion of the cochlear duct the scalae are forming and the mesenchyme is extremely thin and trabeculated. Around the apex of the cochlear duct there are no scalae

On the second day after birth the scalae ves tibuli and tympani have both reached the apex of the cochlear duct (Plate 18 Fig. D). The *helicotrema* is not completely differentiated, but there are only a few strands of tissue separating the scalae. On the fourth day the helicotrema is well formed (Plate 18 Fig. E).

V Development of the Innervation of the Vestibular Apparatus and Cochlear Duct

A Development of the Ganglia of the Eighth Nerve

The seventh and eighth cranial nerve ganglia are represented by one cell mass in the twelve day embryo (*Plate 19 Fig* A) The facial-stato-acoustic ganglion lies medial to the ven tral part of the otocyst The mass of nerve cells splits ventrally into a medial part the acoustic ganglion and a lateral part the geniculate ganglion (Plate 19 Fig B) Fibers from both ganglia penetrate the brain by the twelfth day of gestation

On the thirteenth day of gestation the stato-acoustic ganglion shows internal differentia tion indicating which part will develop into the vestibular ganglion and which will form the spiral ganglion (Plate 19 Fig C) At about the level of the rudimentary posterior semicircular duct, which is ventral to the point at which seventh and eighth ganglia diverge from each other the eighth ganglion is differentiated into lateral and medial portions The medial por tion of the ganglion has nuclei which are more densely packed than the nuclei of the lateral portion The lateral half does not extend as far ventrad as the medial half and represents the forerunner of the vestibular ganglion. The medial half terminates in the loop between the ventral end of the saccule and the newly formed half coil of the cochlear duct. It is the primordium of the spiral ganglion.

On the fourteenth day the spiral ganglion extends under one complete turn of the coch-lear duct (Plate 19 Fig. D) The duct itself has about one and one quarter turns, but no spiral ganglion cells are visible under the last quarter turn. At fifteen days, the seventh and eighth ganglia are still contiguous. There is also no physical separation of the vestibular and the spiral ganglia. Between the sixteenth and seventeenth days, the vestibular and the spiral ganglia have separated considerably In the eighteen day embryo the two ganglia are separate

The geniculate ganglion and the vestibular ganglion are not completely separate until the first day after birth. Until that time there is a sparse accumulation of ganglion cells in the gap between the two ganglia (Plate 19 Fig. E)

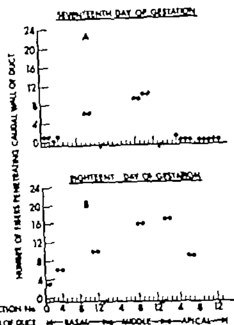
Between the seventeenth and eighteenth days of gestation the cochlear duct attains its full number of coils, and the spiral ganglion ex tends to the apical coil.

B Innervation of the Vestibular Apparatus and Cochlear Duct

The twelfth day otocyst is innervated by fibers from the acoustic ganglion. There are no fibers innervating the otocyst dorsal to the point where the endolymphatic duct flows into it. Ventral to this level the ganglion lies in close proximity to the rostral wall of the otocyst (Plate 20 Fig. A). A bundle of fibers extends laterad from this portion of the ganglion, runs around the rostral end of the otocyst, and penetrates the *rostral part of the lateral* wall. The wall of the otocyst contains three to four layers of nuclei in this region and the nerve fibers penetrate to the innermost layer (Plate 20 Fig. B).

The thirteenth day otocyst is more extensively innervated than the twelfth day otocyst. Most dorsally a bundle of fibers innervates the rostral wall of the otocyst at the level where the endolymphatic duct flows in (Plate 21 Fig. A). Ventral to this a bundle of fibers extends laterad from the ganglion, circumscribes the rostral edge of the otocyst, and innervates its lateral wall (Plate 21 Fig. B). The outermost fibers of the bundle run the longest distance and penetrate the lateral wall opposite the opening of the endolymphatic duct. The inner fibers of the bundle do not extend as far caudad, and they penetrate the wall near the rostral end. The wall contains four to five strata of nuclei and the fibers *penetrate to the innermost stratum* (Plate 21 Fig. C). Ventral to this a third fiber bundle extends from the ganglion to the medial side of the rostral portion of the otocyst (Plate 22, Fig. A). The fibers do not penetrate as deeply and are considerably less numerous than the fibers to the lateral wall (*Plate 22, Fig. B*). The most ventral fiber bundle originates in the ventral end of the ganglion. It extends ventrad and caudad along the medial side of the otocyst to innervate the rudimentary posterior semicircular duct (Plate 22, *Fig. C*).

Those fiber bundles already present on the thirteenth day of gestation are thicker on the fourteenth day. On the fourteenth day they are seen to represent the major fiber bundles of the adult. The most dorsal fiber bundle runs laterad from the ganglion at the level of the macula of the utricle and the lateral semicircular duct (Plate 23 Fig. A). *Some fibers extend dorsad from this bundle to the crista of the superior semicircular duct. The remaining fibers continue laterad. The inner fibers extend only as far as the rostral portion of the macula of the utricle* (*Plate 23 Fig. B*). *The outer fibers continue laterad past the utricle to the crista of the lateral semicircular duct. Ventral to this bundle, in the region of the saccule, bundles of fibers extend from the ganglion to the medial wall of the saccule,*



Graphs 2A and 2B. Nerve fiber penetration of caudal wall of cochlear duct on seventeenth and eighteenth days of gestation. Each cochlear duct studied was considered as being composed of three equal parts: basal, middle and apical portions. Each portion was studied individually. Thirteen equally spaced microscopic sections of each portion, from the beginning to the end of that portion, were analyzed. Using oil immersion (×1 000) and focusing in all planes, the number of nerve fibers penetrating the caudal wall of the duct was counted.

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The geniculate ganglion and the vestibular ganglion are not completely separate until the first day after birth Until that time there is a sparse accumulation of ganglion cells in the gap between the two ganglia (Plate 19 Fig. E)

Between the seventeenth and eighteenth days of gestation the cochlear duct attains its full number of coils, and the spiral ganglion extends to the apical coil.

and Hilding, using phase contrast microscopy have found that the internal tunnel begins to open at about the sixth day postpartum and is complete by the tenth day after birth (8)

The inner and outer hair cells, tunnel of Corti, internal sulcus and limbus spiralis differentiate in the basal coil first, and differentiation proceeds upward toward the apex. This confirms Weibel's observations (20) Kikuchi and Hilding similarly found that the tunnel of Corti begins to open at the basal end and moves upward toward the apex (8)

Kikuchi and Hilding have shown with the electron microscope that differentiation of the stria vascularis proceeds from base to apex and that by ten days after birth the adult pattern is reached (10) Mikaelian and Ruben report that the stria attains normal adult dimensions by the eighth day postpartum (12) Weibel states that by the second day after birth the stria vascularis has reached its final structure (20). He comes to this conclusion by comparing the two-day old ear with that of a nine-day old mouse. It was found in the present study that even at ten days postpartum the stria appears immature. On the tenth day postpartum the stria is still more cellular than the adult stria.

Weibel states that the limbus spiralis is complete by the sixth day postpartum. The present study indicates that the limbus is not fully developed even at ten days postpartum. As early as the fourth day postpartum, all components of the limbus are present. However on the tenth day after birth, the structure is far more cellular than the adult limbus.

Innervation of the sensory areas of the inner ear precedes differentiation of the epithelium. The otocyst is innervated by fibers of the eighth nerve on the twelfth day of gestation. On the thirteenth day of embryonic development the fibers of the eighth nerve have divided up into the fiber bundles which appear to be those characteristic of the adult animal. These include an upper bundle to the cristae of the superior and lateral semicircular ducts and the macula of the utricle, and more ventral fiber bundles to the macula of the saccule and to the crista of the posterior semicircular duct. All of these fiber bundles on the thirteenth day innervate regions which have not differentiated into sensory epithelium. However all three cristae of the semi-circular ducts and the two maculae are recognizable on the fourteenth day On the fourteenth day of gestation the epithelium of the maculae and cristae have clearly differentiated into sensory and supporting cells. Similarly differentiation of the epithelium of the cochlear duct follows the earliest innervation of the duct. When the first half coil develops on the thirteenth day there is no differentiation of the basal epithelium. However fibers from the spiral ganglion already penetrate the cochlear duct epithelium on the thirteenth day

Knowlton (11) has reported that histogenesis of sensory areas in the inner ear of the embryonic chick is correlated with neural development. She reports that the sequence in the sensory area begins with penetration of sensory epithelia by acoustic dendrites. This is followed by a chemical change in the primordial sensory cell cytoplasm, formation of primitive covering membranes, and segregation of sensory and supporting cells. The appearance of a discrete nerve branch in the chick precedes subdivision of a sensory region.

Orr (13) has reported that embryonic chick otocysts and associated tissues cultivated in organ culture developed completely differentiated sensory epithelial membranes of the inner ear Enzymatically dissociated otocysts reassociated and, if they were supported by mesenchyme, developed into sensory membranes composed of hair cells and supporting cells. Cytodifferentiation of hair cells of innervated membranes was complete, whereas differentiation of hair cells in noninnervated membranes was retarded. Innervation was necessary for complete cytodifferentiation of hair cells in reconstructed epithelial membranes.

The fact that nerve fiber penetration precedes differentiation of the epithelial wall in the normal mouse inner ear suggests that the

where the macula is forming (Plate 23 Figs C D) They are not organized into a well formed fiber bundle and the innervation of the saccule is not as dense as that of the utricle. The most ventral fiber bundle runs caudad and ventrad from the ventral end of the vestibular ganglion and innervates the crista of the posterior semicircular duct (Plate 23 Fig. E)

The sensory epithelium of the vestibular apparatus is already innervated on the thirteenth day before differentiation of that epithelium into a macula or a crista occurs.

The epithelium of the caudal wall of the cochlear duct is innervated on the thirteenth day of gestation when only one half coil has formed (Plate 24 Fig. A) The spiral ganglion expands as the cochlear duct develops more coils. On the seventeenth day of gestation the middle portion of the cochlear is most heavily innervated (Graph 2 A) The basal coil is innervated less extensively and the apical coil shows almost no innervation On the eighteenth day of gestation the middle portion is still more heavily innervated than either the apical or basal portions of the duct (Graph 2 B) On subsequent days of development all portions of the cochlear duct are heavily innervated. By the second day after birth it is clear that the fibers innervating the cochlear duct include both radial and longitudinal fibers (Plate 24 Fig. B)

VI Discussion

The membranous labyrinth which is represented by an ovoid sac on the eleventh day of gestation has all of the major components of the adult inner ear when the mouse is born Postnatal development takes the form of cytological differentiation rather than the formation of gross structures.

The semicircular ducts and the cochlear duct both start to develop between the twelfth and thirteenth days of gestation The superior duct forms before the posterior duct. The lateral duct is the latest in developing It has been similarly reported that in the chick the vertical canals are formed before the horizontal canal (13) Between the seventeenth and eighteenth days of gestation the cochlear duct attains its full number of coils. Weibel reports that on the thirteenth day of embryonic development the cochlear duct describes one complete winding (20) However it was found in the present study that on the thirteenth day the cochlear duct had only about one half coil.

The differentiation of sensory and supporting cells begins on the fourteenth day of gestation throughout the cristae, maculae and cochlear duct. Differentiation of the inner and outer hair cells starts on the seventeenth day of gestation Mikaelian and Ruben report that the hair cells appear to be mature by the eighth day postpartum (12) The present study indicates that between the eighth and tenth day the hair cells and phalangeal cells become narrower with the result that spaces are formed between them The hair cells appear mature by the tenth day after birth. Differences such as this are in part accounted for by the different histological techniques used in the preparation of the tissues Mikaelian and Ruben fixed their specimens with 10 formalin and embedded them in celloidin The specimens used in the present study were fixed in 10% Acrolein and embedded in polyester wax

The cupulae of the cristae were not preserved in the tissue sections There was eosinophilic material resembling rudimentary tectorial membrane as early as the sixteenth day of gestation However it was not observed consistently until the first day after birth

The pillar cells and internal tunnel appear complete by the tenth day postpartum. Kikuchi

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VII Summary

This report provides a day by day analysis of the anatomic and histological changes taking place in the inner ear of the normal mouse from the eleventh day of gestation to the tenth day after birth. An atlas of photomicrographs illustrates the developmental processes. The formation of the patterns of innervation of the vestibular apparatus and cochlear duct is discussed It was found that the membranous labyrinth develops all of the major components of the adult inner ear before birth and that postnatal development takes the form of cytological differentiation rather than the formation of gross structures. Differentiation of the cochlear duct proceeds from base to apex. Innervation of the sensory areas of the inner ear precedes differentiation of the epithelium. The possibility that the nerve fibers have an inductive effect on the undifferentiated epithelium is discussed

Zusammenfassung

Dieser Bericht umfasst eine täglich fortlaufende Analyse anatomischer und histologischer Veränderungen des inneren Ohres einer normalen Maus vom 11 Tag der Trächtigkeit bis zum 10 Tag nach der Geburt. Ein Atlas mit Photomikrographien veranschaulicht den Entwicklungsprozess. Die Entstehung der Innervationsrhythmen des Vestibularapparates und des Cochlearganges wurde beschrieben Es wurde festgestellt, dass das Membranlabyrinth die Hauptbestandteile des ausgewachsenen Innerohres vor der Geburt entwickelt und dass die postnatale Entwicklung nur die Form cytologischer Differenzierung und nicht die Form grosser Strukturen einnimmt. Die Differenzierung des Cochlearkanales verlauft von der Basis zur Spitze Die Innervation der sensorischen Gebiete des inneren Ohres geht der Differenzierung des Epithels voraus Die Möglichkeit eines induktiven Effektes der Nervenfasern auf das undifferenzierte Epithel wurde besprochen.

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nerve fibers may play a role in inducing the differentiation of the sensory epithelium in the mouse The work of Knowlton and Orr suggest that this may be true in the chick These relationships among the different elements in the developing inner ear of the mammal could be studied more thoroughly in mouse otocysts grown *in vitro* in the absence of the eighth nerve ganglion

VII Summary

This report provides a day by day analysis of the anatomic and histological changes taking place in the inner ear of the normal mouse from the eleventh day of gestation to the tenth day after birth An atlas of photomicrographs illustrates the developmental processes. The formation of the patterns of innervation of the vestibular apparatus and cochlear duct is discussed. It was found that the membranous labyrinth develops all of the major components of the adult inner ear before birth and that postnatal development takes the form of cytological differentiation rather than the formation of gross structures. Differentiation of the cochlear duct proceeds from base to apex. Innervation of the sensory areas of the inner ear precedes differentiation of the epithelium. The possibility that the nerve fibers have an inductive effect on the undifferentiated epithelium is discussed

Zusammenfassung

Dieser Bericht umfasst eine täglich fortlaufende Analyse anatomischer und histologischer Veränderungen des inneren Ohres einer normalen Maus vom 11 Tag der Trächtigkeit bis zum 10 Tag nach der Geburt Ein Atlas mit Photomikrographien veranschaulicht den Entwicklungsprozess. Die Entstehung der Innervationsrhythmen des Vestibularapparates und des Cochlearganges wurde beschrieben Es wurde festgestellt, dass das Membranlabyrinth die Hauptbestandteile des ausgewachsenen Innerohres vor der Geburt entwickelt, und dass die postnatale Entwicklung nur die Form cytologischer Differenzierung und nicht die Form grosser Strukturen einnimmt. Die Differenzierung des Cochlearkanales verläuft von der Basis zur Spitze. Die Innervation der sensorischen Gebiete des inneren Ohres geht der Differenzierung des Epithels voraus. Die Möglichkeit eines induktiven Effektes der Nervenfasern auf das undifferenzierte Epithel wurde besprochen.

VIII Acknowledgement

The author wishes to express his thanks to the National Institute of Neurological Disease and Blindness for giving him the opportunity to carry out these investigations. The study was initiated at New York University School of Medicine Department of Otorhinolaryngology and completed at the Albert Einstein College of Medicine Department of Otorhinolaryngology The work was carried out in the laboratory of Dr Robert J Ruben whose guidance criticism and aid are gratefully acknowledged Miss Aracelis Colmenares prepared the histological specimens, and Mr Milton Kurtz prepared the photomicrographs. Mr Thomas Van De Water offered many helpful suggestions throughout the study

Appendix

	Endolymphatic duct and sac	Utriculosaccular duct and utriculoendolymphatic valve	Semicircular ducts	Cristae of semicircular ducts
18 G. D.				
19 G. D				Number of layers of supporting cell nuclei has decreased to less than three. In some areas there is only one layer
1 P. P				The cristae has only one layer of supporting cells topped by single layer of sensory cells

Table I

In the following tables, G D refers to days of gestation and P P refers to days postpartum.

	Endolymphatic duct and sac	Utriculosaccular duct and utriculoendolymphatic valve	Semicircular ducts	Cristae of semicircular ducts
12 G D	Endolymphatic duct is present ends blindly consists of simple high cuboidal epithelium			
13 G D			Superior duct is complete, Posterior duct is starting to form and is represented by an evagination from ventral part of caudal end of utricle. No crus commune is present No sign of formation of lateral duct	
14 G D	Blind end of duct has enlarged to form endolymphatic sac Walls of duct and sac consist of simple cuboidal epithelium	None There is wide open channel between utricle and saccule	All 3 ducts are formed. Walls consist of simple cuboidal epithelium except for pseudostratified cuboidal epithelium on convex sides. Crus commune is present. All 3 ducts have ampullae of simple tall cuboidal epithelium	All 3 semicircular ducts have cristae which consist of a stratum of supporting cells with 5 to 6 layers of nuclei topped by a single layer of tall columnar cells
15 G D		Constriction exists in region of endolymphatic duct and is the anlage of the utriculosaccular duct There is Y-shaped confluence of utricle, saccule, and endolymphatic duct Utricle and saccule lie along straight line so utriculossacular duct is linear Utriculoendolymphatic valve is present in rudimentary form		
16 G D				
17 G D		Utriculoendolymphatic valve extends across the opening of the utricle It has converted duct into utriculoendolymphatic and sacculoendolymphatic ducts which meet at a right angle at the origin of the endolymphatic duct Walls of ducts are simple cuboidal cells		Periotic connective tissue and fibers of eighth cranial nerve have pushed up into the cores of the cristae

	Endolymphatic duct and sac	Utriculosaccular duct and utriculoendolymphatic valve	Semicircular ducts	Cristae of semicircular ducts
18 G D				
19 G D				Number of layers of supporting cell nuclei has decreased to less than three. In some areas there is only one layer
1 P P				The cristae has only one layer of supporting cells topped by a single layer of sensory cells

Table II

	Maculae of utricle and saccule	Number of coils of cochlear duct	Formation of ductus reuniens	Differentiation of inner and outer hair and pha langeal cells	Internal tunnel differentiation
12 G D.		0			
13 G D	In region where rostral loop of superior duct flows into utricle the wall has 6 layers of supporting cell nuclei topped by single layer of tall cells, May be anlage of macula of utricle or of crista	1/2	There is none, There is wide open channel between cochlear duct and rest of otocyst		
14 G D	Maculae of utricle and sac cule formed Consist of a stratum of supporting cells with 5 to 6 layers nuclei topped by a single layer of tall columnar cells	1 1/4		Entire caudal wall of cochlear duct consists of a stratum of supporting cells with 5 to 6 layers of nuclei topped by a single layer of columnar cells	
15 G D		1 1/4	Starting to form, Wide channel between cochlear duct and saccule has narrowed. Ductus reuniens forerunner has - layers of nuclei on its lateral side and 5 to 6 layers on its medial side		
16 G D	There is less interdigitation between supporting and sensory cells than on previous days. In some areas there is a gap between top layer of sensory cells and supporting cells below	1 1/2	Lateral wall consists of simple cuboidal epithelium and medial wall consists of pseudostratified cuboidal epithelium. Walls are of simple cuboidal epithelium		
17 G D		1 1/2		The top row of cells in lateral portion of basal 1/3 of cochlear duct show signs of differentiation into 3 outer and	In basal coil, between inner and outer hair cells, is an area of *highly vacuolated* cytoplasm

	Maculae of utricle and saccule	Number of coils of cochlear duct	Formation of ductus reuniens	Differentiation of inner and outer hair and phalangeal cells	Internal tunnel differentiation
				1 inner hair cells. The hair cells rest on a stratum of supporting cells that has 2 rows of nuclei. The hair cell nuclei stand higher than nuclei of adjacent cells	
18 G. D.		1 3/4 (Full number of coils)	Walls are of simple cuboidal epithelium	Basal and middle coils have inner and outer hair cells. They rest on single layer of supporting cells (phalangeal cells)	Basal and middle coils have area of highly vacuolated cytoplasm in region where internal tunnel is forming
19 G. D.				Basal, middle, and apical coils all have inner and outer hair cells. They rest on a single layer of supporting cells	Basal, middle and apical coils have area of vacuolated cytoplasm in region where tunnel is forming
Notes on subsequent days of development				Next day of significant change is the 8th day postpartum. At that time, inner and outer hair cells are becoming narrower and phalangeal cells are vacuolated. Hair cells and phalangeal cells continue to become narrower on the 9th day. By the 10th day postpartum the hair cells are so narrow that the nucleus occupies the entire width of the cell	By the 10th day postpartum the internal tunnel is mature

Table III

Note. The five following structures do not begin to differentiate before the 17th day of gestation

	Pillar cell differentiation	Space of nuel differentiation	Limbus spiralis differentiation	Sulcus spiralis internus differentiation	Tectorial membrane formation
17 G D					
18 G D			*Limbus is present* in basal coil only Axial portion of caudal wall of basal turn has become simple cuboidal epithelium. Beneath it is cellular condensed connective tissue		
19 G D			Limbus is starting to form in middle coil In basal coil the connective tissue under the epithelium is less *cellular than* on the previous day and there is more extra-cellular matrix		T M begins to form. Represented by *thin strip* of eosinophilic material over the cells in region of sulcus spiralis *internus*
1 P P			Limbus is differentiated throughout the cochlear duct except the ex*treme apex*. In the basal and middle coils there is an area *of low cell* density in the connective tissue immediately under the epithelium		
2 P P				The epithelial cells of the caudal wall of the cochlear duct lateral to the limbus are slightly vacuolated	
3 P P					
4 P P			Limbus is complete throughout the cochlear duct It consists of a mound of con-		

	Pillar cell differentiation	Space of Nuel differentiation	Limbus spiralis differentiation	Sulcus spiralis internus differentiation	Tectorial membrane formation
			nective tissue covered by simple tall cuboidal epithelial cells with long thin nuclei. Beneath the epithelium there is wide band of connective tissue of low cell density		
5 P P	The nuclei of the inner and outer pillar cells have moved to opposite sides of the tunnel				
6 P P				The number of layers of nuclei in basal coil in region of sulcus has decreased to 2 or 3 from original 5. The cytoplasm in this region is disintegrating. Middle coil has 2 to 3 layers of nuclei topped by cytoplasm full of vacuoles	
Notes on subsequent days of development	By the 10th day postpartum the pillar cells attain mature form	On the 9th day postpartum spaces form between phalangeal cells. By 10th day Space of Nuel formed		On 7th day P P the apical coil shows much vacuolization of cytoplasm. Basal and middle coils are similar to previous day On 10th day P P sulcus is complete throughout cochlear duct and is lined by layer of simple cuboidal epithelium	

Table IV

	Stria Vascularis differentiation	Reissner's membrane formation	Formation of Scalae Tympani and Vestibuli
12 G D			Mesenchyme around otocyst is condensed, especially adjacent to ventral portion
13 G D		Rostral wall of cochlear duct consists of epithelium with two to three layers of nuclei	Cochlear duct is completely surrounded by mesenchyme that is more condensed than on the previous day
14 G D		Rostral wall has 2 to 3 layers of nuclei but merges into simple columnar epithelium at the extreme medial and lateral edges of the wall	Connective tissue is differentiated into an inner ring and a denser outer ring
15 G D		Large areas of rostral wall of cochlear duct consist of simple cuboidal epithelium	Outermost ring resembles cartilage. Inner ring is loose connective tissue
16 G D		Rostral wall of cochlear duct consists of simple cuboidal epithelium throughout the duct. This represents the inner cell layer of Reissner's membrane	The connective tissue in the inner ring is very sparse and the cytoplasm of those cells forms fine trabeculae
17 G D	A condensation of mesenchyme containing many capillaries develops adjacent to outer part of rostral wall of cochlear duct. Wall of duct in this region consists of simple cuboidal epithelium		
18 G D	Condensed mesenchyme is denser		Scalae tympani and vestibuli *have formed around basal coil*
19 G D	The condensed mesenchyme is denser. The cuboidal epithelial cells in the part of the wall adjacent to the mesenchyme are smaller and their nuclei are darker staining than in adjacent epithelial cells not developing into stria	Parts of rostral wall of basal turn of cochlear duct have two layers. Inner layer is derived from otocyst, outer layer is from mesenchyme	
1 P P	Boundary between epithelial wall and connective tissue is less distinct. Nuclei of epithelial cells are angular and oriented at a right angle to the connective tissue surface. The epithelial cells are dentate with projections into the connective tissue		
2 P P	No clear boundary is discernible between epithelial wall and condensed connective tissue		Scalae tympani and vestibuli have both reached apex of cochlear duct. Helicotrema not completely formed

	Stria Vascularis differentiation	Reissner' membrane *formation*	Formation of Scalae Tympani and Vestibuli
3 P P	The connective tissue cells are more closely packed and their nuclei are smaller and darker staining than previously	Rostral wall of entire basal and middle coils of cochlear duct consists of two layers of cells	Helicotrema is completed
4 P P	There are 2 limiting walls: outer one is mesenchyme and inner one is from otocyst wall. In between are capillaries intermingled with connective tissue cells	Reissner's membrane is complete in all turns of the cochlear duct	

Table IV

	Stria Vascularis differentiation	Reissner s membrane formation	Formation of Scalae Tympani and Vestibuli
12 G D			Mesenchyme around otocyst is condensed especially adja cent to ventral portion
13 G D		Rostral wall of cochlear duct consists of epithelium with two to three layers of nuclei	Cochlear duct is completely surrounded by mesenchyme that is more condensed than on the previous day
14 G D		Rostral wall has 2 to 3 layers of nuclei but merges into simple columnar epithelium at the extreme medial and lateral edges of the wall	Connective tissue is differentiated into an inner ring and a denser outer ring
15 G D		Large areas of rostral wall of cochlear duct consist of simple cuboidal epithelium	Outermost ring resembles cartilage. Inner ring is loose connective tissue
16 G D		Rostral wall of cochlear duct consists of simple cuboidal epithelium throughout the duct. This represents the inner cell layer of Reissner s membrane	The connective tissue in the inner ring is very sparse and the cytoplasm of those cells forms fine trabeculae
17 G D	A condensation of mesenchyme containing many capillaries develops adjacent to outer part of rostral wall of cochlear duct. Wall of duct in this region consists of simple cuboidal epithelium		
18 G D	Condensed mesenchyme is denser		Scalae tympani and vestibuli have formed around basal coil
19 G D	The condensed mesenchyme is denser The cuboidal epithelial cells in the part of the wall adjacent to the mesenchyme are smaller and their nuclei are darker staining than in adjacent epithelial cells not developing into stria	Parts of rostral wall of basal turn of cochlear duct have two layers. Inner layer is derived from otocyst outer layer is from mesenchyme	
1 P P	Boundary between epithelial wall and connective tissue is less distinct Nuclei of epithelial cells are angular and oriented at a right angle to the connective tissue surface. The epithelial cells are dentate with projections into the connective tissue		
2 P P	No clear boundary is discernible between epithelial wall and condensed connec tive tissue		Scalae tympani and vestibuli have both reached apex of cochlear duct. Helicotrema not completely formed


R
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Plate 1

A. Eleventh day of gestation otocyst transected through dorsal portion. 280

B Twelfth day of gestation. ear transected at level of opening of endolymphatic duct 180

C Twelfth day of gestation. ear transected dorsal to opening of endolymphatic duct Orientation is same as in Figure B × 2.0

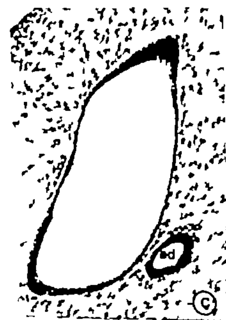
D Twelfth day of gestation. ear transected ventral to opening of endolymphatic duct. Ganglion of eighth nerve lies rostral to ear 180.

Abbreviations (Plates 1–24)

a	apical coil of cochlear duct	mu	macula of utricle
ag	acoustic ganglion	nf	nerve fibers
b	basal coil of cochlear duct	o	otoconia
C	caudad	ohc	outer hair cell
cap	capillary	pd	posterior semicircular duct
cd	cochlear duct	R	rostrad
cld	crista of lateral semicircular duct	rm	Reissner's membrane
ed	endolymphatic duct	rnf	radial nerve fibers
fb	nerve fiber bundle	s	saccule
gc	ganglion cell	sd	superior semicircular duct
h	helicotrema	sg	spiral ganglion
ihc	inner hair cell	sn	sensory cell nuclei
L	laterad	st	scala tympani
ld	lateral semicircular duct	sv	scala vestibuli
lnf	longitudinal nerve fibers	t	Tunnel of Corti
ls	limbus spiralis internus	tm	tectorial membrane
M	mediad	u	utricle
ml	middle portion of cochlear duct	vg	vestibular ganglion


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Plate 1

A. Eleventh day of gestation otocyst transected through dorsal portion. 280.

B Twelfth day of gestation, ear transected at level of opening of endolymphatic duct. 180.

C. Twelfth day of gestation, ear transected dorsal to opening of endolymphatic duct Orientation is same as in Figure B 270

D Twelfth day of gestation, ear transected ventral to opening of endolymphatic duct. Ganglion of eighth nerve lies rostral to ear 180

Abbreviations (Plates 1–24)

a	apical coil of cochlear duct	mu	macula of utricle
ag	acoustic ganglion	nf	nerve fibers
b	basal coil of cochlear duct	o	otoconia
C	caudad	ohc	outer hair cell
cap	capillary	pd	posterior semicircular duct
cd	cochlear duct	R	rostrad
cld	crista of lateral semicircular duct	rm	Reissner's membrane
ed	endolymphatic duct	rnf	radial nerve fibers
fb	nerve fiber bundle	s	saccule
gc	ganglion cell	sd	superior semicircular duct
h	helicotrema	sg	spiral ganglion
ihc	inner hair cell	sn	sensory cell nuclei
L	laterad	st	scala tympani
ld	lateral semicircular duct	sv	scala vestibuli
lnf	longitudinal nerve fibers	t	Tunnel of Corti
ls	limbus spiralis internus	tm	tectorial membrane
M	mediad	u	utricle
mi	middle portion of cochlear duct	vg	vestibular ganglion


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Plate 2

A. Thirteenth day of gestation ear transected through dorsal portion of superior semicircular duct 245

B Thirteenth day of gestation. ear transected through evagination which is anlage of posterior semicircular duct. Material in lumen is artifact. 230.

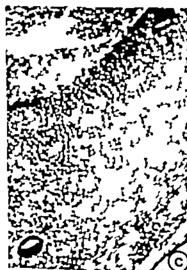
C. Fourteenth day of gestation ear transected through dorsal portion of superior semicircular duct. 140

D Fourteenth day of gestation. ear transected through ampullae and cristae of superior and lateral semicircular ducts. ×280

E. Seventeenth day of gestation. ampulla and crista of superior semicircular duct ×175


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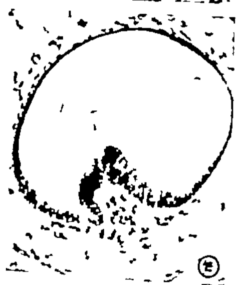

E

Plate 2

A. Thirteenth day of gestation, ear transected through dorsal portion of superior semicircular duct 245

B. Thirteenth day of gestation, ear transected through evagination which is anlage of posterior semicircular duct. Material in lumen is artifact. ×230.

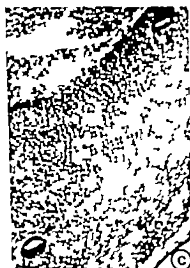
C Fourteenth day of gestation, ear transected through dorsal portion of superior semicircular duct. ×140

D Fourteenth day of gestation, ear transected through ampullae and cristae of superior and lateral semicircular ducts. ×280

E. Seventeenth day of gestation, ampulla and crista of superior semicircular duct. 175


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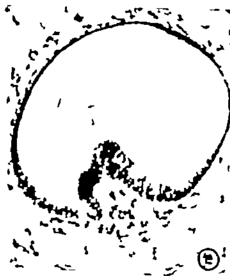

E

Plate 3

A. Fourteenth day of gestation crista of superior semicircular duct. 465

B Seventeenth day of gestation. crista of superior semicircular duct. 465

C. Nineteenth day of gestation. crista of superior semicircular duct. 465

D First day postpartum. crista of superior semicircular duct. × 465


sn
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B


C


D

Plate 4

A. Fifteenth day of gestation ear transected through anlage of utriculosaccular duct, showing utricle saccule and endolymphatic duct. Arrowhead points to rudimentary utriculoendolymphatic valve 175

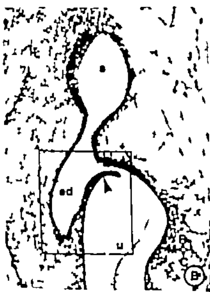
B. Seventeenth day of gestation ear transected through utriculosaccular duct, showing utricle saccule endolymphatic duct and utriculoendolymphatic valve. Arrowhead points to utriculoendolymphatic valve The rectangle outlines the area shown in Figure C at higher magnification. 160

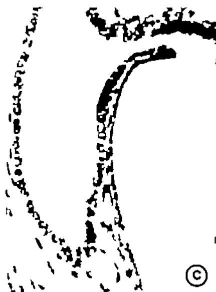
C Seventeenth day of gestation. utriculoendolymphatic valve. × 310.

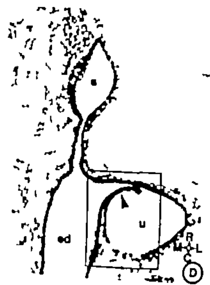
D Nineteenth day of gestation. ear transected through utriculosaccular duct showing utricle saccule endolymphatic duct and utriculoendolymphatic valve. Arrowhead points to utriculoendolymphatic valve. The rectangle outlines the area shown in Figure B at higher magnification. 160.

E. Nineteenth day of gestation. utriculoendolymphatic valve. × 310


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Plate 5

A. Thirteenth day of gestation. ear transected through utricle at point where rostral loop of superior semicircular duct enters utricle. Endolymphatic duct is medial to semicircular duct. Anlage of sensory epithelium is present on rostral wall of utricle (arrowhead). The rectangle outlines the area shown in Figure B at higher magnification. 140.

B Thirteenth day of gestation: Arrowheads point to anlage of sensory epithelium seen in Figure A. 475

C. Fourteenth day of gestation. macula of utricle. 430.


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Plate 5

A. Thirteenth day of gestation. ear transected through utricle at point where rostral loop of superior semicircular duct enters utricle. Endolymphatic duct is medial to semicircular duct. *Anlage of sensory epithelium is present on rostral* wall of utricle (arrowhead). The rectangle out lines the area shown in Figure B at *higher* magnification. 140.

B. Thirteenth day of gestation. Arrowheads point to anlage of sensory epithelium seen in Figure A. 475

C. Fourteenth day of gestation. macula of utricle. × 430


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Plate 6

A. Sixteenth day of gestation. macula of saccule ×505

B Seventeenth day of gestation. macula of saccule. ×505

C Eighteenth day of gestation. macula of saccule ×505

D Nineteenth day of gestation. macula of utricle. The otoconia in this specimen stain lightly possibly due to decalcification 505


L—M
A
L—M
B
L—M
C

Plate 7

A. Fifteenth day of gestation, cross section of ductus reuniens. × 430

B. Sixteenth day of gestation, cross section of ductus reuniens. 430

C. Eighteenth day of gestation, cross section of ductus reuniens. 430.











Plate 8

A Thirteenth day of gestation, cross section of cochlear duct. 290.

B Fourteenth day of gestation, cross section of cochlear duct. Orientation is same as in Figure A × 365

C. Fifteenth day of gestation cross section of middle portion of cochlear duct. Orientation is same as in Figure A. × 400.

D Sixteenth day of gestation mid-modiolar section through cochlear duct. 55

E. Sixteenth day of gestation, cross section of basal coil of cochlear duct Arrowhead points to space in caudal wall of cochlear duct which appears to be artifact. Orientation is same as in Figure A. × 310.


A


B


C


D


E

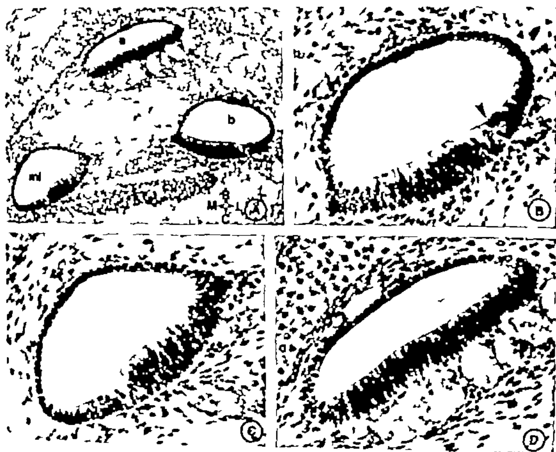
Plate 9

A Seventeenth day of gestation mid-modiolar section of cochlear duct. Arrowhead points to area of differentiation of sensory cells. × 35

B. Seventeenth day of gestation, cross section of basal coil of cochlear duct. Arrowhead points to area of differentiation of sensory cells. 245

C. Seventeenth day of gestation, cross section of middle portion of cochlear duct. 245

D Seventeenth day of gestation, cross section of apical coil of cochlear duct. 45



a
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B
C
D

Plate 10

A. Eighteenth day of gestation, mid-modiolar section through cochlear duct. 40.

B Eighteenth day of gestation, cross section of basal coil of cochlear duct Arrowhead points to area of differentiation of sensory cells. × 80.

C. Eighteenth day of gestation, cross section of middle portion of cochlear duct. Arrowhead points to area of differentiation of sensory cells. 80

D Eighteenth day of gestation, cross section of apical coil of cochlear duct. × 80.


b
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b
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Plate 11

A. Nineteenth day of gestation. mid modiolar section through cochlear duct. 75

B First day postpartum. cross section of middle portion of cochlear duct showing rudiment of tectorial membrane 335

C. First day postpartum cross section of apical coil of cochlear duct × 320


a
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ml
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A


tm
B

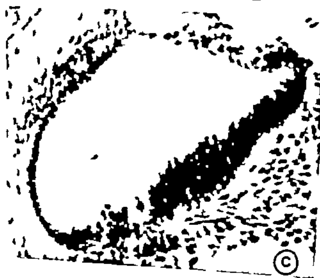

C

Plate 12

A. Fifth day postpartum, caudal wall of apical coil of cochlear duct 350

B Sixth day postpartum caudal wall of apical coil of cochlear duct. 350

C. Eighth day postpartum caudal wall of apical coil of cochlear duct. 350

D Tenth day postpartum, caudal wall of apical coil of cochlear duct 350

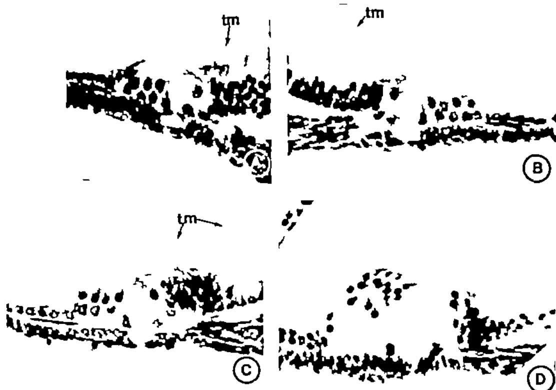

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tm
B
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C
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Plate 12

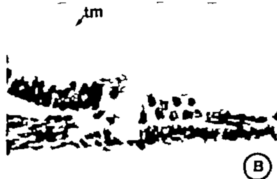
A Fifth day postpartum, caudal wall of apical coil of cochlear duct. 350

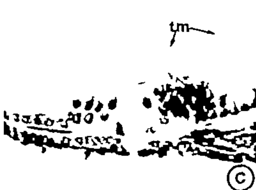
B Sixth day postpartum caudal wall of apical coil of cochlear duct. × 350

C. Eighth day postpartum caudal wall of apical coil of cochlear duct. 350

D Tenth day postpartum caudal wall of apical coil of cochlear duct × 350


tm
A


tm
B


tm
C


D

Plate 13

A. Nineteenth day of gestation cross section of middle portion of cochlear duct, showing anlage of limbus spiralis. 310

B First day postpartum mid modiolar section of cochlear duct. Differentiation of limbus spiralis progresses from base to apex. 45

C First day postpartum. limbus spiralis in cross section of basal coil of cochlear duct. 310

D First day postpartum limbus spiralis in cross section of apical coil of cochlear duct. 310.


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Plate 13

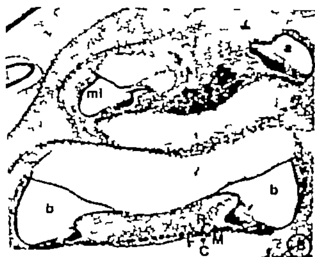
A. Nineteenth day of gestation, cross section of middle portion of cochlear duct, showing anlage of limbus spiralis. × 310

B First day postpartum mid modiolar section of cochlear duct. Differentiation of limbus spiralis progresses from base to apex. × 45

C. First day postpartum limbus spiralis in cross section of basal coil of cochlear duct. 310

D First day postpartum, limbus spiralis in cross section of apical coil of cochlear duct. 310.


A


a
ml
b
b
C
B


D


C

Plate 14

A Second day postpartum. lumbus and region of sulcus spiralis internus of basal coil of cochlear duct. Cytoplasm in region of sulcus is vacuolated. × 275

B Sixth day postpartum mid-modiolar section of cochlear duct. Differentiation of sulcus spiralis internus progresses from base to apex. 35

C Seventh day postpartum. lumbus and region of sulcus spiralis internus of apical coil of cochlear duct Cytoplasm in region of sulcus is vacuolated. 85

D Tenth day postpartum. mid-modiolar section of cochlear duct. Sulcus spiralis is well differentiated throughout cochlear duct. 30


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Plate 15

A Thirteenth day of gestation cross section of cochlear duct Rostral wall is anlage of Reissner s membrane. ×250

B Fourteenth day of gestation. cross section of cochlear duct. Arrowheads point to ep thelial wall which is anlage of Reissner s membrane. Orientation is same as in Figure A. ×300.

C Sixteenth day of gestation cross section of basal coil of cochlear duct. The rostral wall consists of simple cuboidal epithelium. Orientation is same as in Figure A. 300

D Sixteenth day of gestation cross section of apical coil of cochlear duct. The rostral wall consist of simple cuboidal epithelium. Orientation is same as in Figure A. 300.

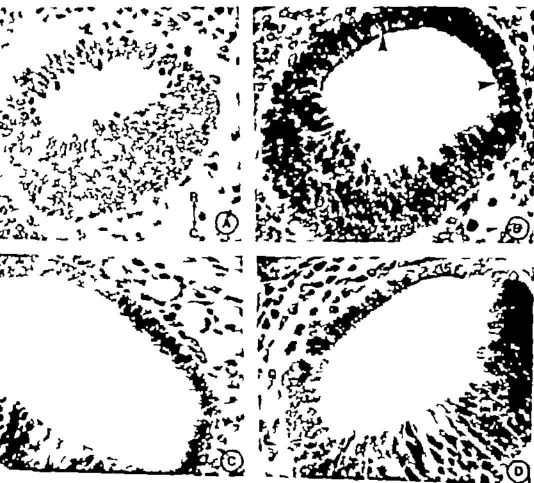

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Plate 15

A Thirteenth day of gestation, cross section of cochlear duct. Rostral wall is anlage of Reissner's membrane. 250

B Fourteenth day of gestation cross section of cochlear duct. Arrowheads point to epithelial wall which is anlage of Reissner's membrane Orientation is same as in Figure A. × 300

C Sixteenth day of gestation, cross section of basal coil of cochlear duct. The rostral wall consists of simple cuboidal epithelium. Orientation is same as in Figure A. 300

D Sixteenth day of gestation, cross section of apical coil of cochlear duct. The rostral wall consists of simple cuboidal epithelium Orientation is same as in Figure A. × 300.

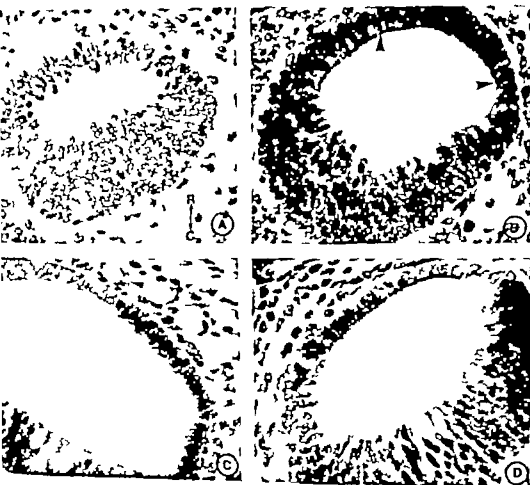

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Plate 16

A. Nineteenth day of gestation cross section of basal coil of cochlear duct. The two cell layers of Reissner's membrane are becoming differentiated. 330

B Nineteenth day of gestation. cross section of apical coil of cochlear duct. The two cell layers of Reissner's membrane are not yet differentiated. × 330.

C Fourth day postpartum. cross section of apical coil of cochlear duct. Reissner's membrane is well differentiated 330


A
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Plate 17

A. Seventeenth day of gestation. anlage of stria vascularis of middle portion of cochlear duct. 350

B Nineteenth day of gestation. anlage of stria vascularis of middle portion of cochlear duct. The cuboidal cells in the part of the wall adjacent to the condensed mesenchyme have nuclei that are slightly smaller and stain considerably more intensely than those of the cells in the adjacent parts of the epithelial wall which are not developing into stria. 280

C First day postpartum. anlage of stria vascularis of middle portion of cochlear duct. 350

D Second day postpartum. anlage of stria vascularis of middle portion of cochlear duct. × 350

E. Fourth day postpartum stria vascularis of middle portion of cochlear duct. 350.




B


C


D


E

Plate 17

A Seventeenth day of gestation anlage of stria vascularis of middle portion of cochlear duct. × 350

B Nineteenth day of gestation, anlage of stria vascularis of middle portion of cochlear duct. The cuboidal cells in the part of the wall adjacent to the condensed mesenchyme have nuclei that are slightly smaller and stain considerably more intensely than those of the cells in the adjacent parts of the epithelial wall which are not developing into stria. 280

C. First day postpartum anlage of stria vascularis of middle portion of cochlear duct. 350

D Second day postpartum, anlage of stria vascularis of middle portion of cochlear duct. 350.

E. Fourth day postpartum stria vascularis of middle portion of cochlear duct. 350




B


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D


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Plate 17

A. Seventeenth day of gestation anlage of stria vascularis of middle portion of cochlear duct. 350.

B Nineteenth day of gestation. anlage of stria vascularis of middle portion of cochlear duct. The cuboidal cells in the part of the wall adjacent to the condensed mesenchyme have nuclei that are slightly smaller and stain considerably more intensely than those of the cells in the adjacent parts of the epithelial wall which are not developing into stria. 280

C *First day postpartum anlage of stria vascularis* of middle portion of cochlear duct. × 350.

D Second day postpartum anlage of stria vascularis of middle portion of cochlear duct. × 350

E. Fourth day postpartum. stria vascularis of middle portion of cochlear duct. × 350.


A


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Plate 18

A. Fourteenth day of gestation. Mesenchyme surrounding membranous labyrinth is differentiated into two concentric rings. × 65

B Fifteenth day of gestation. outermost ring of mesenchyme surrounding membranous labyrinth is beginning to resemble cartilaginous tissue. 60.

C. Eighteenth day of gestation. mid-modiolar section of cochlear duct. Scalae vestibuli and tympani are differentiated around basal coil. × 45

D Second day postpartum mid-modiolar section of cochlear duct Scalae vestibuli and tympani have formed up to apex. Helicotrema is not completely differentiated (arrowhead). × 35

E. Fourth day postpartum. mid modiolar section of cochlear duct. Helicotrema is completely differentiated. × 35


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Plate 19

A. Twelfth day of gestation. facial-acoustic ganglion. Protargol. × 375

B Twelfth day of gestation. acoustic ganglion and geniculate ganglion ventral to the point of bifurcation. Protargol. 230.

C. Thirteenth day of gestation. The acoustic ganglion is differentiating internally into anlagen of vestibular and spiral ganglia. Protargol 280.

D Fourteenth day of gestation. The spiral ganglion extends under one turn of the cochlear duct, but not under the most apical quarter turn. Protargol. × 335

E. Nineteenth day of gestation. Isolated ganglion cells lie along fibers between geniculate and vestibular ganglia. × 300




B



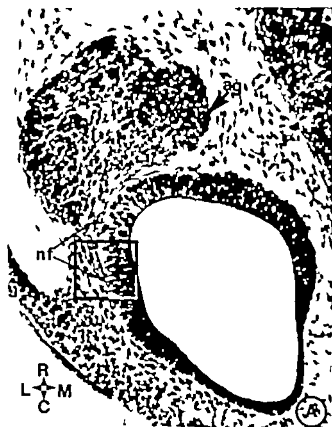




Plate 20

A Twelfth day of gestation. Nerve fibers from acoustic ganglion extend to lateral wall of otocyst The rectangle outlines the area shown in Figure B at higher magnification. Protargol. ×400

B. Twelfth day of gestation. Nerve fibers penetrate lateral wall of otocyst Arrowheads point to nerve fibers. Protargol. 1 000 oil.


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Plate 21

A. Thirteenth day of gestation most dorsal fiber bundle (arrowheads). Arrowheads point to nerve fibers. Protargol 290

B Thirteenth day of gestation. second most dorsal fiber bundle (arrowheads) The rectangle outlines the area shown in Figure C at higher magnification Protargol × 160.

C. Thirteenth day of gestation. Fibers of the second most dorsal fiber bundle penetrate the lateral wall of the otocyst Protargol. 860, oil


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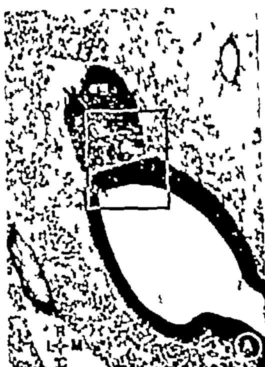

C

Plate 22

A Thirteenth day of gestation The third most dorsal fiber bundle extends from the acoustic ganglion to the rostromedial wall of the otocyst. The rectangle includes the area shown in Figure B at higher magnification. Protargol 140.

B Thirteenth day of gestation. Fibers of the third most dorsal bundle penetrate the rostro-medial wall of the o ocyst (arrowheads). Protargol. 870 oil

C. Thirteenth day of gestation. most ventral fiber bundle. Protargol. 350.


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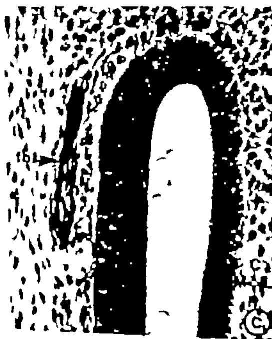

C

Plate 23

A. Fourteenth day of gestation, most dorsal fiber bundle Nerve fibers extend to crista of lateral semicircular duct and macula of utricle. The rectangle includes the area shown in Figure B at higher magnification. Protargol. 265

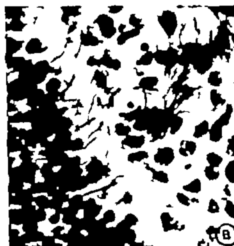
B Fourteenth day of gestation Fibers of most dorsal bundle penetrate macula of utricle Protargol 700, oil

C Fourteenth day of gestation. Nerve fibers innervate medial wall of saccule. The rectangle includes the area shown in Figure D at higher magnification. Protargol × 265

D Fourteenth day of gestation. Nerve fibers penetrate medial wall of saccule Protargol. 700 oil

E. Fourteenth day of gestation. Most ventral fiber bundle innervates crista of posterior semicircular duct Protargol. 315


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Plate 23

A. Fourteenth day of gestation most dorsal fiber bundle. Nerve fibers extend to crista of lateral semicircular duct and macula of utricle. The rectangle includes the area shown in Figure B at higher magnification. Protargol. 265

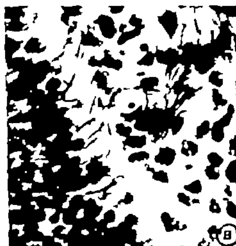
B Fourteenth day of gestation. Fibers of most dorsal bundle penetrate macula of utricle Protargol 700 oil.

C Fourteenth day of gestation. Nerve fibers innervate medial wall of saccule. The rectangle includes the area shown in Figure D at higher magnification. Protargol 265

D Fourteenth day of gestation. Nerve fibers penetrate medial wall of saccule. Protargol. 700, oil.

E. Fourteenth day of gestation. Most ventral fiber bundle innervates crista of posterior semicircular duct. Protargol. 315


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C
A


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C


D


E

Plate 23

A. Fourteenth day of gestation, most dorsal fiber bundle. Nerve fibers extend to crista of lateral semicircular duct and macula of utricle. The rectangle includes the area shown in Figure B at higher magnification Protargol 65

B. Fourteenth day of gestation. Fibers of most dorsal bundle penetrate macula of utricle Protargol × 700, oil.

C Fourteenth day of gestation. Nerve fibers innervate medial wall of saccule. The rectangle includes the area shown in Figure D at higher magnification. Protargol. × 265

D Fourteenth day of gestation. Nerve fibers penetrate medial wall of saccule. Protargol. 700, oil.

E. Fourteenth day of gestation. Most ventral fiber bundle innervates crista of posterior semicircular duct. Protargol 315


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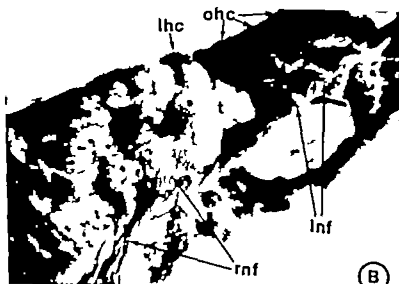

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Plate 24

A. Thirteenth day of gestation. Fibers of the acoustic ganglion innervate the caudal wall of the cochlear duct. Protargol 4 0

B Second day postpartum. cross section through organ of Corti Radial and longitudinal nerve fibers are present Protargol 1 000 oil

Acta

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SUPPLEMENT 286

Fluorescence and Electron Microscopic Observations of the General Visceral, Efferent Innervation of the Inner Ear

BY

MURIEL D ROSS

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STOCKHOLM, SWEDEN

ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 286

Fluorescence and Electron Microscopic Observations of the General Visceral, Efferent Innervation of the Inner Ear[1,2]

BY

MURIEL D ROSS

From the Department of Anatomy
The University of Michigan
Ann Arbor Michigan 48104, USA

UPPSALA 1971

Introduction

The basic anatomy of the autonomic innervation of the inner ear is not well understood and is even a matter of considerable dispute. This situation exists in spite of the fact that knowledge of the autonomic innervation patterns could conceivably lead to a better understanding of fundamental influences on the normal hearing process and, thus, to better interpretations of causes underlying some diseases of the inner ear including Ménière's syndrome and some forms of deafness. That at least the sympathetic system might influence the sensory input at the periphery has been indicated by physiological experiments which showed that stimulation of cervical sympathetics results in decreased cochlear microphonics (Seymour & Tappin, 1953) and that transection of fibers of the stellate ganglion results in increased microphonics (Beickert et al. 1956).

Some anatomical support for the physiological findings appeared with the fluorescent results of Spoendlin and Lichtensteiger (1966), who reported that the inner ear was supplied with adrenergic fibers of dual origin and distribution. One group of fibers, according to these observers, formed a perivascular plexus around the labyrinthine artery and its major branches within the modiolus. The other more significant group of fluorescing fibers, distributed with the cochlear nerve fibers rather than with the blood vessels. This latter group of fibers ended in a plexus within the osseous spiral lamina near the habenula perforata, where it was predicated, the norepinephrine released could affect the sensory fibers at the site of the generator potential. Spoendlin (1966) published electron micrographs which supported the finding of such a terminal distribution. Spoendlin & Lichtensteiger (1967) later reported experimental results which indicated that the adrenergic fibers to the inner ear were of dual origin as well as of dual distribution, the perivascular fibers were said to arise from the lower cervical ganglia, the fibers among the acoustic nerve fibers, from the superior cervical ganglion.

The earlier findings of Spoendlin & Lichtensteiger (1966) were quickly opposed by Terayama et al. (1966) who, using essentially the same fluorescent method, found a similar distribution of fluorescent fibers but said that all the fibers were derived from the perivascular plexus and all were related to blood vessels, even those near the habenula. The adrenergic innervation was interpreted by this group of investigators as entirely related to fluid balance. Terayama, working with different collaborators (Terayama et al., 1968), later carried out experiments in which the superior cervical ganglion was extirpated on one side and then, after a time, the inner ears of both sides were examined electron microscopically in order to ascertain localization of degenerating fibers. Results of this study were interpreted as largely confirming the previous findings of Terayama et al. (1966) that the adrenergic system had but one source, the superior cervical ganglion, and that it was almost exclusively related to blood vessels. This new study, however, led to the disclosure that a few fibers with granular vesicles in them were not related to blood vessels. Some of these fibers were found at the habenula and remained normal under the experimental conditions. These fibers were interpreted as belonging largely to the "olivocochlear" bundle.

It was mostly in an attempt to resolve some of the controversy over the source and ultimate distribution of the adrenergic fibers to the inner ear that the present work was begun. Fluorescent results obtained from mouse, rat,

[1] This research has been supported by grant NB-07306
from the United States Public Health Service

[2] A preliminary report of some of the findings included
here was presented at the meeting of the American
Association of Anatomists in Boston, Massachusetts, April, 1969

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Introduction

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Observations

Fig. 1 is a drawing made from a thick section (1 μ) of the cochlea of the rat stained with toluidine blue and used for orientation purposes for ultra-thin sectioning. This figure should be consulted for localizing the various portions of the cochlea referred to in the text.

Labyrinthine artery

The number of fluorescing fibers along the labyrinthine artery is not great in any of the forms studied (Fig. 2) In the kitten, the fluorescing fibers appear continuous with the perivascular plexus of the basilar-anterior inferior cerebellar arteries. In the mouse and rat this continuity is not so obvious. In the latter forms, the fluorescing fibers along the labyrinthine artery and its major branches appear as the artery positions itself under the eighth nerve the basilar and anterior inferior cerebellar arteries have few fluorescent fibers associated with them.

In the rat and mouse, study of serial sections demonstrates that fluorescing fibers follow a route from the carotid plexus, along the continuation of the internal carotid artery within the skull, through a small foramen into the facial canal, and along the facial nerve from its genu back toward the brain stem. At the internal acoustic meatus, the fibers position themsel es in the arachnoid mater and split into two fascicles which are directed caudally and rostrally around the cochle r nerve The exact further course of these fibers could not be determined conclusively in the fluorescent material the fibers appeared to join the labyrinthine artery and tra el with its main branches, the A estibuli anterior and the A. cochleae communis (terminology followed is that of Smith, 1951)

In all form in est gated, the main branches of the A cochleae communis within the modiolus are supplied with adrenergic fibers as are some eins, especially in the kitten and dog (Fig. 3). In the dog, there are large, green fluorescent structures associated with the smaller arteries located among the branching cochlear nerve fibers (Fig. 4). Whether these are fluorescent cell bodies of neurons, or simply bundles of adrenergic fibers, could not be determined.

The green fluorescent beaded fibers of the perivascular plexuses terminate within the modiolus. Furthermore, in none of the many sections studied have fluorescing fibers been observed diverging from the labyrinthine artery

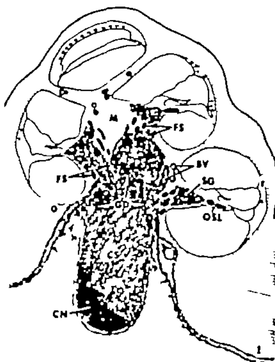


Fig. 1 This drawing, made from a thick (1 μ) section of the rat cochlea used for orientation purposes for lectron microscopy should be consulted for localization of anatomical structures or regions referred to in the text. *BV* blood essels; *CN* cochlear nerve; *CS*, central segment of the cochlear nerve; *FS*, foramina of the spiral tract; *GD* glial dome of the cochlear nerve, separating central and peripheral segments of the nerve; *M* modiolar bone; *OSL*, osseous spiral lamina; *FS*, peripheral segment of the cochlear nerve; *SG* spiral ganglion.

cat and puppy form the main basis of the report presented here Most recently investigation of the localization of unmyelinated fibers in various portions of the inner ears of these same forms at the level of the electron microscope has been initiated in order to better determine the relationship of such fibers to the vascular system. Although the latter work, when completed will be published separately pertinent data already obtained from one form, the rat is included here.

Materials and Methods

This report is based upon fluorescence microscopic investigations of serial sections obtained from 28 mice and 45 albino rats (Sprague Dawley) ranging in age from 17–26 days and 10 kittens and 1 puppy which were newborn. Electron microscopic examination of cochleae from 13 rats ranging from 17 days to 2 months in age was also carried out. The complete technique used in preparing the tissue for fluorescence microscopy has been published elsewhere (Ross, 1969 *b*) Briefly all animals were decapitated and after removal of lower jaw fur and extraneous tissue the head with inner ears *in situ* was quick frozen on dry ice while being sprayed with Cryokwik (International Equipment Company) The tissue was mounted and sectioned at 14 μ in a cryostat at 20 C and serial sections were mounted on warm slides. All sections were stored in the cryostat in glass staining dishes containing a little silica gel and when sectioning was completed, were allowed to dry in the cryostat overnight (about 16 hours) with the anti fog fan running In the morning the slides were transferred to Coplin jars filled to one third capacity with paraformaldehyde which had been previously stored over 54% sulfuric acid The jars were sealed and placed in an 80 C oven for periods ranging from 15 min to 3 hours, with 1 hour incubation time the usual case. Sections were allowed to cool then mounted in immersion oil and studied with a Leitz Ortholux fluorescent microscope. A 2 mm KG 1 heat filter and a 3 mm UG 1 filter were used as excitation filters Barrier filters of 510 and 530 nm were employed and dark field illumination was used exclusively All photomicrographs shown here were taken using the 530 nm barrier filter on TRI X Pan Film (Kodak) utilizing 1 $^{1}/_{2}$ min exposure time.

Eleven of the rats prepared for electron microscopy were anesthetized with chloral hydrate (1 ml/100 g body weight) injected intraperitoneally and then perfused for about 20 min with glutaraldehyde-paraformaldehyde (Karnovsky 1965) in 0.2 M sodium-cacodylate HCl buffer Entire cochleae were removed and placed in ethylenedinitrilotetraacetic acid glutaraldehyde (Baird et al 1967) for 3 weeks for decalcification Tissues were post fixed in 1% OsO_4 in 0 144 M sodium-cacodylate HCl buffer to which 0 15 CaCl had been added, dehydrated in a graded series of alcohols, and embedded in Epon The remaining 2 rats used for electron microscopy were prepared in a similar way except that a Krebs Ringer phosphate buffer (Van Orden et al 1970) was substituted for the cacodylate buffer This procedure was followed in the hope of preserving membrane bound granules in the adrenergic fibers. Sections 1–2 μ thick were cut and stained with toluidine blue for orientation purposes, after which the block was trimmed and ultra-thin sections were cut Sections were mounted unsupported on 200 mesh grids or on single hole grids covered with Formvar membranes stained with lead citrate (Reynolds, 1963), and studied on a RCA EMU 3 G electron microscope.

Observations

Fig. 1 is a drawing made from a thick section (1 μ) of the cochlea of the rat stained with toluidine blue and used for orientation purposes for ultra-thin sectioning. This figure should be consulted for localizing the various portions of the cochlea referred to in the text.

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In the rat and mouse, study of serial sections demonstrates that fluorescing fibers follow a route from the carotid plexus, along the continuation of the internal carotid artery within the skull, through a small foramen into the facial canal, and along the facial nerve from its genu back toward the brain stem. At the internal acoustic meatus, the fibers position themselves in the arachnoid mater and split into two fascicles which are directed caudally and rostrally around the cochlear nerve. The exact further course of these fibers could not be determined conclusively in the fluorescent material; the fibers appeared to join the labyrinthine artery and travel with its main branches, the A. vestibuli anterior and the A. cochleae communis (terminology followed is that of Smith, 1951).

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The green fluorescent beaded fibers of the perivascular plexuses terminate within the modiolus. Furthermore, in none of the many sections studied have fluorescing fibers been observed diverging from the labyrinthine artery

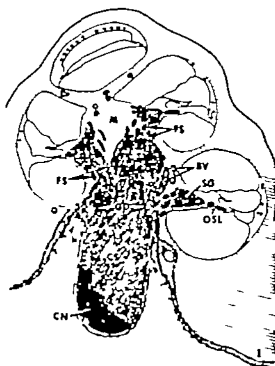


Fig. 1. This drawing, made from a thick (1 μ) section of the rat cochlea used for orientation purposes for electron microscopy, should be consulted for localization of anatomical structures or regions referred to in the text. BV, blood vessels; CN, cochlear nerve; CS, central segment of the cochlear nerve; FS, foramina of the spiral tract; GD, glial dome of the cochlear nerve, separating central and peripheral segments of the nerve; M, modiolar bone; OSL, osseous spiral lamina; PS, peripheral segment of the cochlear nerve; SG, spiral ganglion.

cat and puppy form the main basis of the report presented here Most recently investigation of the localization of unmyelinated fibers in various portions of the inner ears of these same forms at the level of the electron microscope has been initiated in order to better determine the relationship of such fibers to the vascular system Although the latter work when completed will be published separately pertinent data already obtained from one form, the rat, is included here.

Materials and Methods

This report is based upon fluorescence microscopic investigations of serial sections obtained from 28 mice and 45 albino rats (Sprague Dawley) ranging in age from 17–26 days and 10 kittens and 1 puppy which were newborn Electron microscopic examination of cochleae from 13 rats ranging from 17 days to 2 months in age was also carried out The complete technique used in preparing the tissue for fluorescence microscopy has been published elsewhere (Ross 1969 *b*) Briefly all animals were decapitated and after removal of lower jaw fur and extraneous tissue the head with inner ears *in situ* was quick frozen on dry ice while being sprayed with Cryokwik (International Equipment Company) The tissue was mounted and sectioned at 14 μ in a cryostat at 20 C and serial sections were mounted on warm slides. All sections were stored in the cryostat in glass staining dishes containing a little silica gel and when sectioning was completed were allowed to dry in the cryostat overnight (about 16 hours) with the anti fog fan running In the morning the slides were transferred to Coplin jars filled to one third capacity with paraformaldehyde which had been previously stored over 54% sulfuric acid. The jars were sealed and placed in an 80 C oven for periods ranging from 15 min to 3 hours, with 1 hour incubation time the usual case. Sections were allowed to cool, then mounted in immersion oil and studied with a Leitz Ortholux fluorescent microscope A 2 mm KG 1 heat filter and a 3 mm UG 1 filter were used as excitation filters. Barrier filters of 510 and 530 nm were employed, and dark field illumination was used exclusively All photomicrographs shown here were taken using the 530 nm barrier filter on TRI X Pan Film (Kodak) utilizing 1 $^1/_2$ min exposure time.

Eleven of the rats prepared for electron microscopy were anesthetized with chloral hydrate (1 ml/100 g body weight) injected intraperitoneally and then perfused for about 20 min with glutaraldehyde paraformaldehyde (Karnovsky 1965) in 0 2 M sodium-cacodylate HCl buffer Entire cochleae were removed and placed in ethylenedinitrilotetraacetic acid-glutaraldehyde (Baird et al. 1967) for 3 weeks for decalcification Tissues were post fixed in 1% OsO_4 in 0 144 M sodium-cacodylate HCl buffer to which 0 15 CaCl had been added dehydrated in a graded series of alcohols, and embedded in Epon The remaining 2 rats used for electron microscopy were prepared in a similar way except that a Krebs Ringer phosphate buffer (Van Orden et al. 1970) was substituted for the cacodylate buffer This procedure was followed in the hope of preserving membrane-bound granules in the adrenergic fibers. Sections 1–2 μ thick were cut and stained with toluidine blue for orientation purposes after which the block was trimmed and ultra thin sections were cut Sections were mounted unsupported on 200 mesh grids or on single hole grids covered with Formvar membranes, stained with lead citrate (Reynolds, 1963) and studied on a RCA EMU 3 G electron microscope.

Observations

Fig. 1 is a drawing made from a thick section (1 μ) of the cochlea of the rat stained with toluidine blue and used for orientation purposes for ultra-thin sectioning. This figure should be consulted for localizing the various portions of the cochlea referred to in the text.

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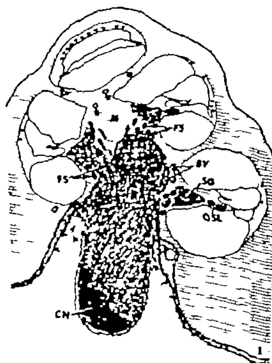


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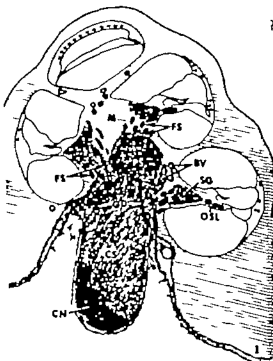


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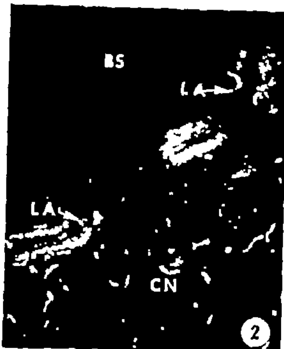








Fig 2 Labyrinthine artery kitten. Only a few fluorescent fibers are associated with the labyrinthine artery which has thin walls The artery (*LA*) is shown here as it curves between the brain stem (*BS*) and cochlear nerve (*CN*) which contains fluorescent fibers. 120
Fig 3 Branches of the spiral modiolar artery (single arrow) and vein (double arrows) dog Fluorescent fibers are associated with both arterial and venous spiral modiolar vessels in all forms studied but are most numerous in the dog. × 120
Fig 4 Spiral modiolar artery proximal to the vessel shown in Fig. 3 Fluorescent structures (arrows) occur along the larger arterial branches to the inner ear in the dog. Whether these structures are small, fluorescent cells or simply bundles of fluorescing fibers could not be determined in the present material. 120
Fig 5 Fluorescent fibers of the cochlear nerve, kitten. The fluorescent fibers appear abruptly in the peripheral segment (*PS*) of the cochlear nerve immediately distal to the glial dome (arrows) The central segment of the cochlear nerve (*CS*) contains only a few scattered, fluorescent structures. 116

or its branches to enter the substance of the cochlear nerve Thus, the perivascular plexus associated with the major cochlear vessels appears to be separate from the adrenergic system of fibers running within the cochlear nerve.

Cochlear nerve

A marked difference in background fluorescence exists between the *central* and *peripheral segments* of the acoustic nerve in the forms studied, so that the glial dome is sharply demarcated (Fig. 5). The perikarya of neurons of the acoustic nerve nucleus, with associated *orange* or *green fluorescence*, lie central to the dome in the mouse and rat (Ross, 1969 *b*). In the kitten, a few fine fluorescent beaded fibers and scattered fluorescent granules occur in the central segment. Observations on the central portion of the acoustic nerve of the dog have been too limited to present an adequate description of fluorescence.

In all cases, green fluorescing fibers, often in thick bundles, appear abruptly at the glial dome running in the peripheral segment of the cochlear nerve. The bundles of fluorescing fibers course peripheralward, joining the fascicles of acoustic nerve fibers at the foramina of the spiral tract (Fig. 6). They enter the *foramina* and continue peripheralward to Rosenthal's canal. At the light microscope level, the fibers appear to be intermingled with acoustic nerve fibers and not particularly related to blood vessels.

Electron microscopic observations of this same portion of the acoustic nerve indicate that all of the blood vessels within the nerve are capillaries or postcapillary venules, containing no smooth muscle in their walls. The capillaries have been classified according to the criteria presented by Rhodin (1968) as venous or arterial in the accompanying micrographs. None of the capillaries are fenestrated. The vessels are enclosed by perivascular sheaths of pia-like cells in which occasional openings occur (Fig. 7).

Proximal to the foramina of the spiral tract, unmyelinated fibers run among acoustic nerve fibers or within a few micra of the perivascular sheaths (Fig. 7). All the unmyelinated fibers observed have been enclosed to some degree in Schwann cell cytoplasm and are never intimately associated with the cells of the perivascular sheath or with the endothelial cells of the capillaries. Within the foramina of the spiral tract, the bundles of acoustic nerve fibers are frequently devoid of capillaries. Unmyelinated fibers occur intermingled with the myelinated in these bundles.

Spiral canal

The green fluorescing beaded fibers, some still in small bundles, interweave in part in a plexus immediately proximal to the spiral ganglion cells. Although some fibers cross the spiral ganglion to reach the intraganglionic spiral bundle and the root of the osseous spiral lamina in all the forms studied (Figs. 8 and 9), such crossing fibers are most numerous in the rat and mouse. In the kitten, many of the fibers travel around the ganglion by passing close to the bony walls of the spiral canal and then run toward the root of the osseous spiral lamina. On rare occasion, some of the crossing fibers become closely associated with, or appear to partly coil about, the perikarya of spiral ganglion cells (Fig. 9). No fluorescent perikarya have been observed in the spiral ganglion of any form studied.

Electron microscopy confirms the fact that unmyelinated fibers are abundant between the spiral ganglion cells but are infrequently situated near the capillaries or postcapillary venules (Fig. 10). The vessels are again enclosed by perivascular sheaths which are, however, usually incomplete.

Osseous spiral lamina

A plexus of fluorescent fibers occurs at the root of the osseous spiral lamina (Fig. 11) in the region of the intraganglionic spiral bundle. In the rat and mouse *particularly some* of the fluorescing fibers run with the latter bundle. In horizontal sections, the *fluorescent fibers* project peripheralward in closely parallel array (Figs. 11 and 12) and *form a plexus* with arcades (Fig. 12) in the region of the inner spiral vessel. When viewed in vertical sections, the *fluorescent fibers* course predominantly along the tympanic *face of the* lamina near the inner spiral vessel and also along the upper

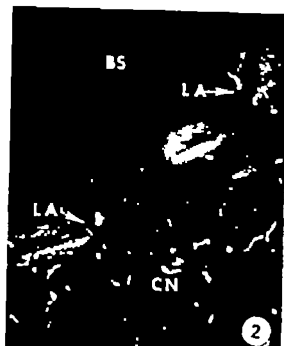








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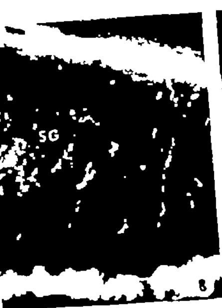
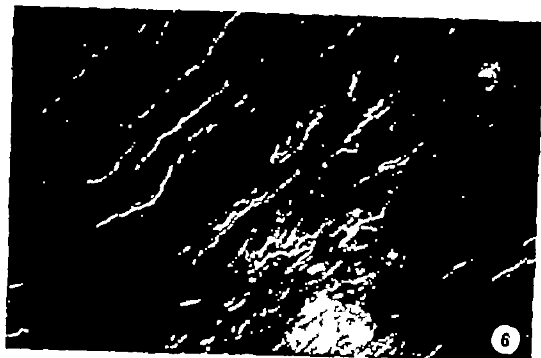




Fig. 8. Oblique section through the spiral ganglion, basal cochlear turn, kitten. Fluorescing fibers traverse the spiral ganglion (SG) singly or in small bundles. 170.

Fig. 9. Horizontal section through the spiral ganglion (SG), middle third of the cochlea, kitten. Fluorescing fibers occasionally closely approach and appear to coil about spiral ganglion cells (arrow). A portion of the plexus in the osseous spiral lamina is indicated by double arrows. 175

Fig. 10. Electron micrograph of unmyelinated fibers (arrow heads) among spiral ganglion cells, middle third of the cochlea, rat. Thinly myelinated fibers (TM) occur near the unmyelinated fibers in this micrograph. Unmyelinated fibers exist singly as well as in fascicles among the spiral ganglion cells. 3500.



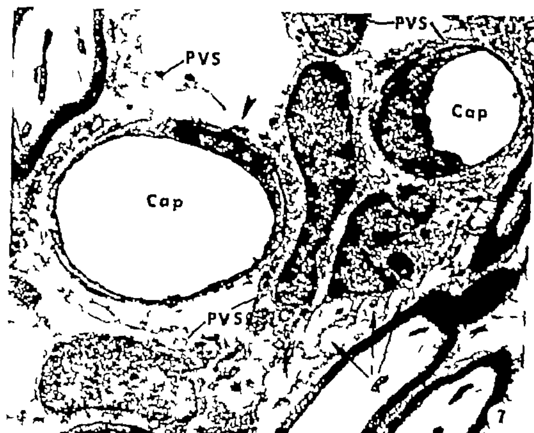


Fig 6 Fluorescent fibers in the branches of the cochlear nerve proximal to the foramina of the spiral tract kitten. Fluorescing fibers ppear to course with acoustic nerve fibers. 120

Fig 7 Electron micrograph of capillaries and acoustic nerve f bers just distal to the glial dome, rat. In this *instance, unmyelinated fibers (arrows) travel close to* the capillaries (*Cap*) which are enclosed in perivascular sheaths (*PVS*). The larger vessel at left center may be a venous capillary and the smaller vessel at upper right, an arterial capillary An opening in the sheath around the vessel at left center is indicated by an *arrow head. Although lying close to the capillaries,* the unmyelinated fibers are coursing in the same direc*tion as the adjacent myelinated, cochlear nerve fibers. (SC* Schwann cell) × 500.

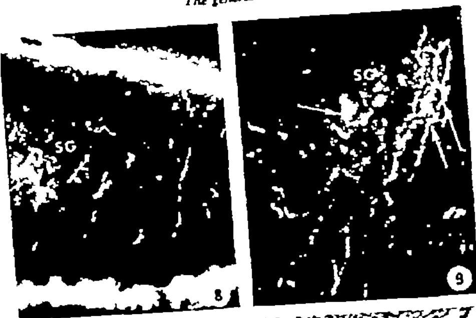
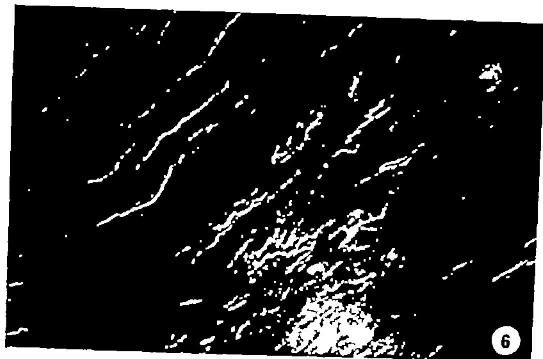


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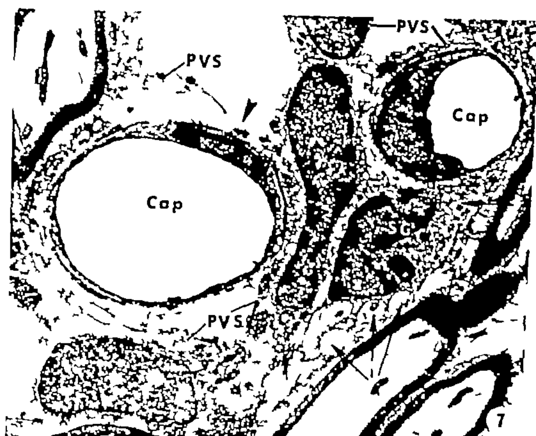


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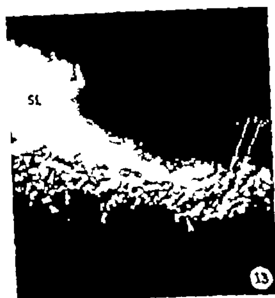




Fig. 13. Vertical section through the distal portion of the osseous spiral lamina, apical third of the cochlea, kitten. Numerous fluorescent fibers are present in the nerve bundles within the osseous spiral lamina. Many of the fluorescent fibers are in small fascicles (arrow heads). The arrows indicate the terminal beads of single fibers in the foramina nervosa. Portions of the spiral limbus (*SL*) are autofluorescent. ×120.

Fig. 14. Slightly oblique section through the habenula perforata, middle third of the cochlea, kitten. Several terminal beads, each in a foramen nervosum, occur between the arrows. Each foramen appears to receive a single terminal. The arrow heads indicate some of the fluorescent fibers, usually in small fascicles, of the peripheral plexus in the osseous spiral lamina. (*SL*, spiral limbus.) ×170.

plate of bone where capillaries course (Fig. 13). A plexus with arcades connects the fluorescing fibers in the region of the inner spiral vessel. Additionally, in the present fluorescent material, there are fibers which terminate as end beads in the foramina nervosa (Figs. 13 and 14). These terminals are most conspicuous in the kitten. None of the green fluorescent fibers have been traced into the organ of Corti. The general pattern of distribution of the fluorescent, adrenergic fibers appeared to be similar in all turns of the cochlea in the species studied.

Examination of sections from the rat osseous spiral lamina with the electron microscope shows that unmyelinated fibers increase tremendously in number at the root of the osseous spiral lamina (Fig. 15), where they course, for the most part, in the intraganglionic spiral bundle. The latter bundle consists of unmyelinated, thinly myelinated, and thickly myelinated fibers. Many of the unmyelinated fibers presumably end within the bundle. This assumption is based upon the fact that the more peripherally coursing bundles of acoustic nerve fibers contain fewer unmyelinated fibers than would be expected on the basis of the numbers present at the root.

Blood vessels with thin walls and an incomplete investment of pericytes cross the region of the intraganglionic spiral bundle at the root of the osseous spiral lamina as they travel toward the modiolar bone. These vessels, on the basis of the ultrastructure of their walls, might be classified as venous capillaries (*Rhodin, 1968*). However, on the basis of their location and large size the vessels correspond to postcapillary venules. The dilated portions ("beads") of beaded, unmyelinated fibers sometimes are associated with these vessels (Fig. 16).

The acoustic nerve fibers traverse the osseous spiral lamina in bundles which run between bony trabeculae. The bundles themselves are devoid of capillaries and are comprised of myelinated nerve fibers, some unmyelinated



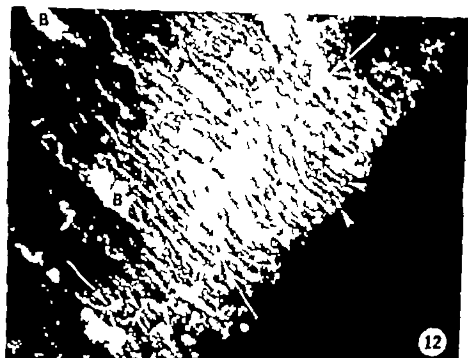


Fig 11 Horizontal section through the piral ganglion *(SG)* and *osseous spiral lamina, middle third of the* cochlea, k tten This photomicrograph demonstrates *the increase in numbers of fluorescent f bers which* occurs at the root of the osseous spiral lamina (arrow heads) The arrows indicate fluorescent fibers which course directly out of the piral ganglion and into the osseous spiral lamina The *fluorescent fibers in the spiral ganglion appear to lie singly or in small fascicles* among the spiral ganglion cells. Bone is autofluores cent *(B)* 1 0

F g 12 Plexus of fluorescent fibers in the distal por*tion of the osseous spiral lamina middle third of the* cochlea, kitten. The fluorescent f bers frequently inter twine *to form arcades (arrow) although the general* direction of the fibers is radial Two terminal end beads, similar to those shown in the foramina nervosa in Figs. 13 and 14 re indicated by arrow heads. *(B autofluorescent bone)* 1 0





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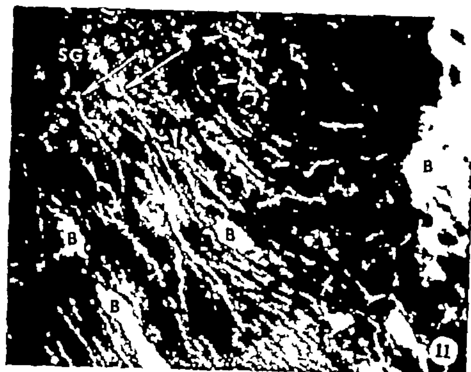
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plate of bone where capillaries course (Fig. 13). A plexus with arcades connects the fluorescing fibers in the region of the inner spiral vessel. Additionally in the present fluorescent material, there are fibers which terminate as end beads in the foramina nervosa (Figs. 13 and 14). These terminals are most conspicuous in the kitten. None of the green fluorescent fibers have been traced into the organ of Corti. The general pattern of distribution of the fluorescent, adrenergic fibers appeared to be similar in all turns of the cochlea in the species studied.

Examination of sections from the rat osseous spiral lamina with the electron microscope shows that unmyelinated fibers increase tremendously in number at the root of the osseous spiral lamina (Fig. 15) where they course, for the most part, in the intraganglionic spiral bundle. The latter bundle consists of unmyelinated, thinly myelinated, and thickly myelinated fibers. Many of the unmyelinated fibers presumably end within the bundle. This assumption is based upon the fact that the more peripherally coursing bundles of acoustic nerve fibers contain fewer unmyelinated fibers than would be expected on the basis of the numbers present at the root.

Blood vessels with thin walls and an incomplete investment of pericytes cross the region of the intraganglionic spiral bundle at the root of the osseous spiral lamina as they travel toward the modiolar bone. These vessels, on the basis of the ultrastructure of their walls, might be classified as venous capillaries (Rhodin 1968). However on the basis of their location and large size, the vessels correspond to postcapillary venules. The dilated portions ("beads") of beaded, unmyelinated fibers sometimes are associated with these vessels (Fig. 16).

The acoustic nerve fibers traverse the osseous spiral lamina in bundles which run between bony trabeculae. The bundles themselves are devoid of capillaries and are comprised of myelinated nerve fibers, some unmyelinated



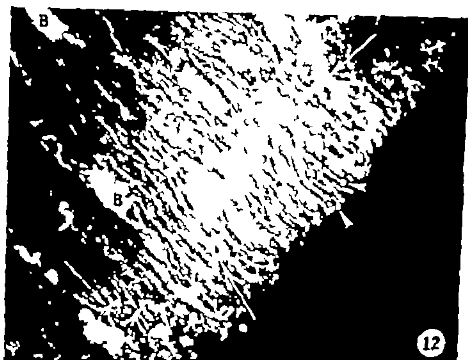


Fig 11 Horizontal section through the spiral ganglion (*SG*) and osseous spiral lamina middle third of the cochlea, kitten. This photomicrograph demonstrates the increase in numbers of fluorescent fibers which occurs at the root of the osseous spiral lamina (arrow heads) The arrows indicate fluorescent fibers which course directly out of the spiral ganglion and into the osseous spiral lamina. The fluorescent fibers in the spiral ganglion appear to lie singly or in small fascicles among the spiral ganglion cells. Bone is autofluorescent (*B*) 120

Fig 12 Plexus of fluorescent fibers in the distal portion of the osseous spiral lamina, middle third of the cochlea, kitten. The fluorescent fibers frequently intertwine to form arcades (arrow) although the general direction of the fibers is radial Two terminal end beads, similar to those shown in the foramina nervosa in Figs. 13 and 14 are indicated by arrow heads. (*B* autofluorescent bone.) 1 0.

fibers, and Schwann cells. In the better preserved material extracellular space diminishes and becomes minimal most peripheralward. Collagen fibrils occur in the narrow extracellular spaces.

The unmyelinated fibers are located among the myelinated fibers or at the periphery of the bundles of nerve fibers. Those fibers at the periphery are sometimes closely related to the perivascular sheaths of the postcapillary venules or venous capillaries which run between the nerve bundles. Rarely unmyelinated fibers have been observed lying within 750–1 000 Å of cell membranes of pericytes of postcapillary venules traveling between the nerve bundles. In one such case, the perivascular sheath was not present and the Schwann cell covering was attenuated (Fig 17).

The blood vessels will be described elsewhere in greater detail, however it should be stressed here that, in the rat at least, none of the vessels in the osseous spiral lamina possess smooth muscle in their walls. All of them are either capillaries or postcapillary venules.

Discussion

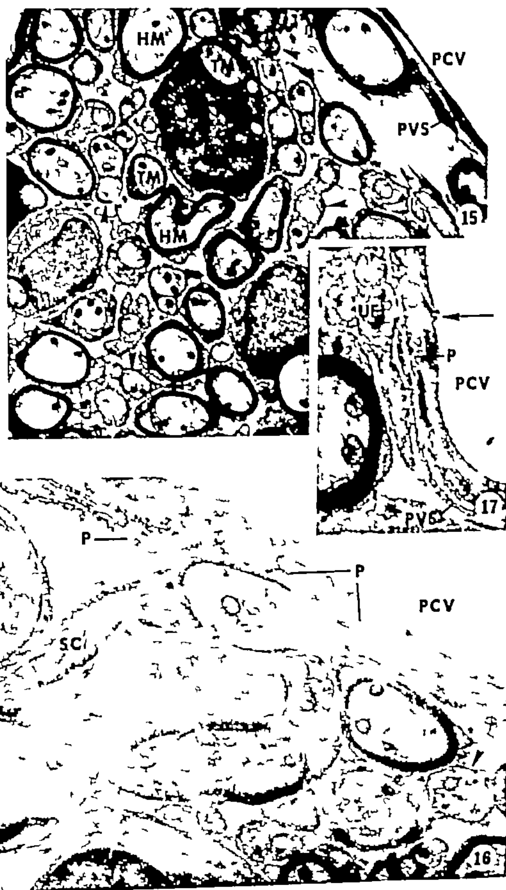
Anatomical results of the present study are in general agreement with the previously published findings of Spoendlin (1966) and Spoendlin & Lichtensteiger (1966, 1967). In concurrence with these authors and in contrast to the findings of Terayama et al. (1966, 1968), there appears to be a dual distribution of adrenergic fibers in the inner ear: one group of fibers is perivascular and the other usually courses independently of blood vessels among acoustic nerve fibers.

It has not been possible in the present anatomical material to establish conclusively whether or not the two groups of fibers are of entirely separate origins. Some adrenergic fibers reach the labyrinthine artery from the basilar plexus, in the rat and mouse, this contribution appears to be minor. A second, more significant, group of fluorescent fibers reaches the inner ear by way of a facial anastomosis. The route taken by this group of fibers is from the carotid plexus to the facial nerve along a course corresponding to that of the greater superficial petrosal nerve then along the facial nerve to the arachnoid mater near the cochlear nerve. This connection could be traced in its entirety in fluorescent material only in the rat and mouse and, in these forms, the further course of the fibers appeared to be along the labyrinthine artery. However, some of the fibers may have turned into the peripheral segments of the acoustic or vestibular nerves.

Fig 15 Cross section of the intraganglionic spiral bundle, middle third of the cochlea, rat. This electron micrograph demonstrates the fact that the intraganglionic spiral bundle contains unmyelinated (arrow heads), thinly myelinated (*TM*), and heavily myelinated (*HM*) nerve fibers. The blood vessel (*PCV*) appears to be a postcapillary venule. (*PVS* perivascular sheath.) 3 900.

Fig 16 An electron micrograph of an unmyelinated fiber lying close to the wall of a postcapillary venule (*PCV*), middle third of the cochlea, rat. Although the unmyelinated fiber is sectioned through an enlargement, or "bead" the Schwann cell (*SC*) still completely envelops the fiber. The sheaths of other unmyelinated fibers (arrow heads at lower right), located farther from the postcapillary venule, are either attenuated or lacking. (*P* pericyte.) 12 600.

Fig 17 Unmyelinated fiber close to the pericyte of the wall of a postcapillary venule in the osseous spiral lamina, middle third of the cochlea, rat. In this instance, a small fascicle of unmyelinated fibers approaches the outer cell membrane of a pericyte (*P*). The unmyelinated fiber (*UF*) closest to the membrane of the pericyte (*P*) has an attenuated Schwann cell covering; no perivascular sheath (*PVS*) intervenes. Overlap of the endothelial cells of the postcapillary venule (*PCV*) is indicated by the arrow. 5 800.


HM
TM
TM
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PVS
17
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16

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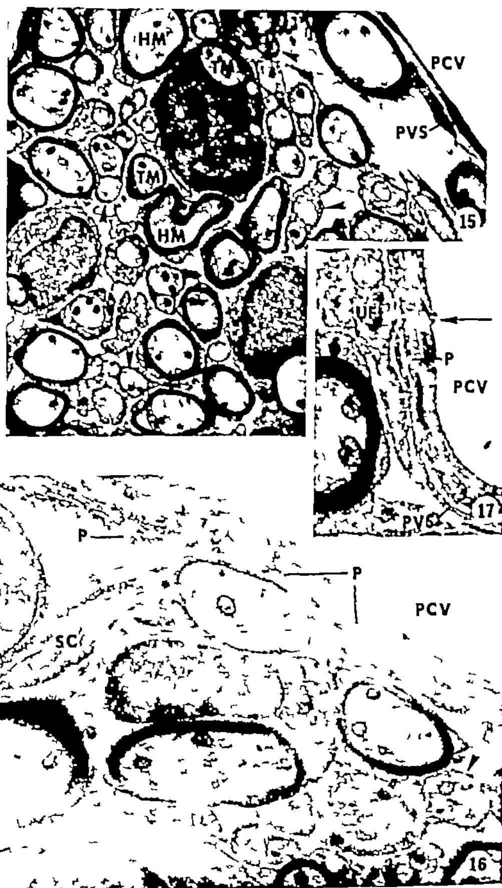
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Fig. 1 Unmyelinated fiber close to the pericyte of the wall of a postcapillary venule in the osseous spiral lamina, middle third of the cochlea, rat. In this instance, small fascicle of unmyelinated fibers approaches the outer cell membrane of a pericyte (*P*). The unmyelinated fiber (*UF*) closest to the membrane of the pericyte (*P*) has an attenuated Schwann cell covering; no perivascular sheath (*PVS*) intervenes. Overlap of the endothelial cells of the postcapillary venule (*PCV*) is indicated by the arrow 5 800.


HM
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PCV
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16

the *inner ear* indicating that they are concerned with a general rather than a specific and highly localized function. However the exact nature of this *generalized function* remains to be physiologically elucidated for the inner ear. Terayama et al. (1966, *1968*) and Spoendlin & *Lichtensteiger* (1966), *observing essentially the same* adrenergic plexus in the inner ear reached entirely different conclusions concerning its functional significance, as has already been pointed out. However both conclusions may be partially correct.

With the finding that fluorescent terminals extend into the *foramina nervosa*, the possibility that the *adrenergic system can influence acoustic nerve* activity at the site of generation of the action potential as predicated by Spoendlin & Lichtensteiger (1966) is on firm anatomical ground. Whether this effect is facilitatory or inhibitory can only be speculated upon in the absence of physiological or pharmacological data. *In the case of* Pacinian corpuscles, where fluorescent fibers have been observed (Santini, 1969) and where primary catecholamines ha e *been shown* to occur (Chunchkov 1968), topical application of norepinephrine or epinephrine results in enhancement of response of the receptor to stimulation (Loewenstein & Altamirano-Orrego 1956). Chernetski (1964) has found that sympathetic stimulation enhances peripheral sensory afferent activity in the frog and has hypothesized that a vertebrate arousal system dependent upon sympathetic outflow *may* exist. Thus, previous experimental results point to the possibility *of enhancement of auditory* nerve activity by the adrenergic nervous system.

However the fibers projecting into the foramina nervosa, demonstrated by fluorescent microscopy here for the first time, represent only a portion of the *adrenergic plexus* in the osseous spiral lamina. The *remaining fibers*, which form arcades and lie below those to the foramina, are the fibers actually described in the prior reports of both Spoendlin & Lichtensteiger (1966, *1967*) and Terayama et al. (1966). These latter fibers are considered here to be possibly related to regulating *fluid* balance within the cochlea. *Although this* interpretation is *in partial agreement* with Terayama et al. (1966, 1968), present conclusions differ in salient respects from theirs.

The anatomical evidence suggests that the adrenergic regulation of fluid balance in the cochlea is achieved in a two-fold way: (1) by regulation of total blood flow into the cochlea through the sympathetic innervation of the labyrinthine artery and *its major branches* within the *modiolus*, and (2) by *regulation of fluid* balance between the intra and extravascular fluids through the peripheral vascular bed. Functionally related to the latter control is the adrenergic innervation of the larger veins leaving the modiolus.

The *peripheral regulatory effects of nor*epinephrine would appear to be of far greater significance to *inner ear function* than the *adrenergic control* of the *labyrinthine artery* when the great numbers of fluorescing fibers extending radialward in the osseous spiral lamina are compared with the small numbers of fluorescing fibers along the artery. Although electron microscopic studies indicate that some of the unmyelinated fibers in the nerve bundles of the osseous spiral lamina may lie within 750–1 000 Å *of pericytes or* perivascular sheaths of postcapillary venules, *most of these* fibers course among the myelinated fibers of the bundles. Thus, release of norepinephrine can occur some distance from the capillary bed into the surrounding tissue fluid. Acoustic nerve fibers, capillaries, and even the perikarya *of the spiral ganglion* may at time, or always, exist and function *in a milieu* rich in norepinephrine.

No close apposition (200 Å) of unmyelinated fibers with endothelial cells of capillaries, as reported by Terayama et al. (1968) to occur in the guinea pig, has been observed in the rat. This could be due to species variation.

The actual *site or sites* in the inner ear which are under the *influence* of norepinephrine can only be determined *experimentally*

Spoendlin & Lichtensteiger (1967) working with cats, have presented experimental evidence that this group of fibers distributes among the acoustic nerve fibers.

The connection from the pericarotid plexus to the facial nerve by way of the greater superficial petrosal nerve as described here on the basis of fluorescent results, was known to earlier anatomists (Pleschel, 1844 in horse Arnold, 1851 in man and calf Henle 1872, in man Penzo 1893 in man cat rat mouse and several other forms Rauber & Kopsch 1909 in man and others) More recently it was described by Chorobski & Penfield (1932, in monkey and man) who found that at least part of the fibers of the anastomosis were parasympathetic and were directed from nervus intermedius to the carotid plexus Some of the earlier investigators indicated that this connection carried sympathetic fibers back toward the facial nerve (Arnold, 1851 Penzo 1893 and others) Arnold and Penzo described the further course of the sympathetic fibers as along the facial nerve back toward the internal acoustic meatus and into the vestibular division of the eighth cranial nerve That the last named authors did not confuse the sympathetic anastomosis with known connections of nervus intermedius appears certain for they also described direct motor fibers of nervus intermedius into the greater superficial petrosal nerve and an anastomosis of nervus intermedius with the vestibular nerve

Thus, early and more recent results (Spoendlin & Lichtensteiger 1967) as well as the present fluorescent findings, indicate that the carotid plexus makes an important communication with the inner ear structures by way of a facial anastomosis. The course taken by the fibers to the facial nerve is probably subject to individual variation Spoendlin & Lichtensteiger (1967) indicated that in the cats used in their studies, the carotid plexus was linked to the seventh cranial nerve by anastomoses with the tenth cranial nerve or with the tympanic plexus.

An interesting difference between the rodents and the other forms used in this study has been the presence of fluorescing multipolar neurons in the central segment of the cochlear nerve in the rodent The histochemical and ultrastructural properties of these neurons have been published (Ross, 1969 b Ross & Burkel, 1970) and will not be taken up again here However these neurons resemble autonomic cells in many ways and it is possible that in the rodent they contribute to the adrenergic innervation of the inner ear

The distribution of the cochlear adrenergic fibers is among ordinary acoustic nerve fibers and spiral ganglion cells almost exclusively according to present fluorescent and electron microscopic findings. These results concur with those of Spoendlin & Lichtensteiger (1966) but are in disagreement with Terayama et al (1966 1968)

The presence of unmyelinated or fluorescent fibers among myelinated nerve fibers in nerve roots or among the perikarya of neurons in sensory ganglia is not peculiar to the acoustic nerve and its ganglion A similar distribution occurs in the vestibular ganglion (personal observations) and has been reported for the fifth cranial nerve and its ganglion in the cat after fluorescent studies (Santini 1966) and electron microscopic investigations (Dixon, 1963) Fluorescent fibers have also been observed in the cat dorsal root ganglion (Owman & Santini, 1966) and rat ventral roots (Dahlström & Fuxe 1965) Occasionally the fluorescent fibers of the Gasserian ganglion and of the dorsal root ganglia were found to coil about the neuronal perikarya as was observed here in the case of the spiral ganglia The possibility exists that the acetylcholinesterase positive fibers observed coiling about spiral ganglion cells previously (Ross 1969 a) might also be adrenergic Numerous recent studies have shown that acetylcholinesterase is associated in varying amounts with a wide variety of both sensory and motor neurons (see Nachmansohn 1970 for review)

The fluorescent adrenergic fibers interweave extensively as they course peripheralward in

the inner ear indicating that they are concerned with a general rather than a specific and highly localized function. However the exact nature of this generalized function remains to be physiologically elucidated for the inner ear. Terayama et al. (1966, 1968) and Spoendlin & Lichtensteiger (1966), observing essentially the same adrenergic plexus in the inner ear reached entirely different conclusions concerning its functional significance, as has already been pointed out. However both conclusions may be partially correct.

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The peripheral regulatory effects of norepinephrine would appear to be of far greater significance to inner ear function than the adrenergic control of the labyrinthine artery *when the great numbers of fluorescing fibers* extending radialward in the osseous spiral lamina are compared with the small numbers of fluorescing fibers along the artery. Although electron microscopic studies indicate that some of the unmyelinated fibers in the nerve bundles of the osseous spiral lamina may lie within 750–1 000 Å of pericytes or perivascular sheaths of postcapillary venules, most of these fibers course among the myelinated fibers of the bundles. Thus, release of norepinephrine can occur some distance from the capillary bed into the surrounding tissue fluid. Acoustic nerve fibers, capillaries, and even the perikarya of the spiral ganglion may at time, or always, exist and function in a milieu rich in norepinephrine.

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The actual site or sites in the inner ear which are under the influence of norepinephrine can only be determined experimentally

Norepinephrine may affect the caliber or permeability of the local capillaries, although there is presently no evidence that norepinephrine has any effect upon blood vessels lacking smooth muscle in their walls On the other hand, norepinephrine released into the tissue fluid of the nerve bundles of the osseous spiral lamina could be carried into the capillaries and to the cells they nourish, to exert an influence upon their activity

It is proposed here that the adrenergic innervation of the cochlea is dual in function, affecting both auditory nerve activity at the site of the generator potential and the fluid balance. A third possibility that the fibers chemodynamically influence the sensory neurons, cannot be discounted at the present time. The existence of the adrenergic system in the inner ears of newborn kittens and puppies, when the sensory system is undergoing final development (Pujol & Marty 1970 also personal observations) and is presumably *nonfunctional* may indicate the existence of some trophic effect upon the growth of the acoustic neuronal dendrites or upon the organ of Corti itself On the other hand, the completeness of the adrenergic system in the newborn animals might be but a reflection of the critical importance of norepinephrine in maintaining the proper fluid medium in the inner ear at the time the organ of Corti is undergoing final development.

Summary

Fluorescence microscopic studies of the cochleae of the rat mouse kitten and puppy indicate that fluorescent adrenergic fibers are of dual distribution in the inner ear One group of fibers is perivascular to the major labyrinthine vessels and the other *more significant* group appears to course chiefly among cochlear nerve fibers and spiral ganglion cells. The latter group occurs in greatest numbers within the osseous spiral lamina where the fibers course in parallel array peripheralward. Some of the fluorescing fibers form arcades others project into the foramina nervosa where they end as terminal beads. Electron microscopic investigation of the rat cochlea supports the fluorescent results. Some unmyelinated fibers approach within 750–1 000 Å of membranes of pericytes associated with capillaries or post capillary venules in the root of or within the osseous spiral lamina but many course independently of the vessels. These results are interpreted as indicating a generalized function for norepinephrine in influencing fluid balance and in modifying auditory nerve activity at the periphery

Zusammenfassung

Fluoreszenzmikroskopische Studien der Schnecke bei Ratte Maus, Katzchen, und Hündchen zeigen dass die fluoreszierenden adrenergischen Fasern des Innenohrs in zwei Gruppen verteilt sind. Die eine Gruppe läuft perivaskulär an den grösseren Blutgefässen des Labyrinthes entlang, die andere bedeutendere Gruppe hat unter den Nervenfasern und zellen des Ganglion spirale ihren Verlauf Diese Gruppe kommt *hauptsächlich in der*

lamina spiralis ossea vor wo die Fasern in parallelen Bündeln gegen die Periferie verlaufen. Einzige fluoreszierende Fasern bilden Arkaden, während andere in den Foramina nervosa als *spheridae terminales* endigen. Elektronmikroskopische Untersuchungen der Schnecke der Ratte bestätigen die Fluoreszenz ergebnisse. Einzige myelinfreie Fasern laufen in nächster Nähe (750–1 000 A) der Grenzmembranen von Perioyten der Kapillargefässe oder postkapillare Venülen der Lamina ossea, viele aber sind ganz unabhängig von den Blutgefässen. Diese Ergebnisse werden als Zeugnis einer Allgemeinfunktion des Noradrenalins, das Flüssigkeitsgleichgewicht im Innenohr zu beeinflüssen und die Aktivität der Hörnerven zu modifizieren.

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by

C. J. H. REIJNEN and W. KUIJPERS

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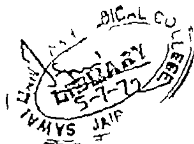
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SUPPLEMENTUM 287

THE HEALING PATTERN OF THE DRUM MEMBRANE

BY

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CONTENTS

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INTRODUCTION

The spontaneous healing of a perforation in the drum membrane depends on the way it is caused and on the size of the defect. Small perforations which originate as a consequence of a trauma or result from an acute otitis media, usually heal rather rapidly. However large traumatic perforations tend to heal in the beginning, but the activity of the healing process gradually declines and often ceases *completely before the perforation* is closed. A perforation originating from a chronic otitis, may vary in size during the healing process, while no complete closure occurs. In these cases the main number of small perforations heals spontaneously after the disappearance of the inflammatory process, the larger ones however rarely.

The evolution in the techniques of closing a persisting perforation in the drum membrane can be divided into two periods. In the first period (1640–1952) starting with the first reported attempt of Banzer a great number of different methods were used to eliminate the harmful consequences of a persisting perforation in the drum membrane, initially with prostheses and afterwards with methods for the purpose of getting the perforation closed. The second period (1952 until now) is characterized by the entrance (Von Moritz, Wullstein and Zöllner) and further development of plastic surgical techniques to get functional reconstruction of the hearing organ. In this period successful healing of drum perforations was obtained with tissues of very different origin. However until now there has been *a large divergency in opinions as to the* usefulness of the tissues that had to be used in tympanic grafting. Moreover fundamental data on the healing process of a defect in the drum membrane and the effect of covering the defect with a graft are extremely scarce.

The present study was undertaken with the object of obtaining more insight in the wound-healing of the drum membrane. The first part of this monograph consists of a critical analysis of the methods of closing a persisting perforation in the drum membrane used until now. In the second part the results of an experimental study on the healing pattern of a traumatic perforation in the drum membrane with and without the use of a graft are described.

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CHAPTER I

ANALYSIS OF THE LITERATURE ON MYRINGOPLASTY

As already mentioned the historical development of the techniques used to eliminate the harmful effect of a persisting perforation in the drum membrane can be divided into two periods 1640–1952 and from 1952 until now Nearly all techniques used in the first period (reviewed by Schrimpf 1954 and Storrs, 1966) have been abandoned. Only the etching of the edges of the perforation with chemical caustics such as trichloracetic acid (Dunlap & Schuknecht, 1947) or silver nitrate are occasionally used at present to close small perforations. The effect of this procedure, which may be compared with the mechanical removal of the edges of a perforation performed as part of the surgical procedure in the period from 1952 until now is of fundamental importance to obtain healing of the perforation. When the inflammatory processes are healed the edges of a persisting perforation are avascular and consist only of strands of dense connective tissue covered with a thin layer of epithelium. Removal of this tissue (mechanically or with chemical caustics) will create a situation comparable with a fresh traumatic perforation. I this way the membrane is stimulated to proliferate. With the use of this method alone, the drum membrane will heal in a few cases only To obtain a higher score it is necessary to cover the perforation with a graft as may be concluded from the results obtained in the second period.

This period started with the introduction of the tympanoplastic surgery a surgical technique to obtain a functional reconstruction of the middle ear and the drum membrane (Von Moritz, 1952 Wullstein, 1952 Zöllner 1952). For the reconstruction of the drum membrane (myringoplasty) they used skin, as was previously used by Berthold (1878), Ely (1881) and Tangemann (1884) and afterwards by Shulbof and Valdez (1944).

The usefullness of skin for myringoplasty was already being discussed a few years after 1952 and afterwards many tissues have been used in tympanic grafting. From the variety of tissues used since that time (reviewed by Örtegren, 1964 and Salén, 1968) and still used it appears that there is no general view on the preferred tissue. This is most likely caused by the lack of sufficient experimental data on the behaviour and the fate of these tissues and their effect on the course of the healing process of the drum membrane. Moreover the arguments used by several authors in favour of a particular tissue are often obscure and founded insufficiently

In considering the present state of affairs it appears that skin, except meatal skin, is no longer used in myringoplasty The properties of the external body skin as drum membrane graft are such that sooner or later chance of failure is very high. Inflammation and excessive desquamation of the graft very often occur These phenomena are highly likely due to the change in the environmental conditions for the graft. The temperature and the humidity near the drum membrane (Nadel & Horvath, 1970) are much higher than at the retro-auricular region, from which the grafts usually originate. Since a full thickness skin graft is rather thick and does not grow much thinner even after several years (Guilford et al., 1965) less improvement in hearing will be obtained (Allen & Fernandez, 1960 Pesavento, 1960). Besides this, the chance of the development of a cholesteatoma in the

used in fact. Only homologous tissues stored at low temperatures, freeze dried or preserved with chemical agents have been used and these are consequently avital. Therefore there is no fundamental difference between these tissues and the membrane of Cargile used by Fox (1945). For that reason it is more correct to talk about implants. A striking phenomenon, however is that no real transplantation reactions were described. Since lyophylisation or preservation of tissues at 0 C (Cornish & Scott, 1966) do not destroy the antigenic properties, disappearance of these properties was only observed if tissues were preserved at temperatures below -20 C (Andresen et al., 1956). Although no data are available, it seems likely to assume that tissues preserved in sterilising fluids (merthiolate, cialit, merfen, ethanol) lose at least part of their antigenic capacity. Besides this, several reasons may be supplied to explain the absence of reactions suggestive of transplantation phenomena in using homologous tissue in myringoplasty.

1. The amount of transplanted tissue is so small that only a slight, subliminal quantity of antibodies is formed (dosage phenomenon of Medawar (1944).

2. The T-antigen, responsible for the transplantation reaction is confined to the nucleus. The homologous tissues used in myringoplasty are mainly composed of fibrous tissue containing only a few cells.

3. The amount of antibodies formed is dependent on the degree of vascularization of the acceptor site. In myringoplasty the initial contact between graft and drum membrane is very small. Moreover it may be supposed that the transplanted tissue might lose a large amount of its antigenic properties before complete integration into the membrane had been established. Therefore the concentration of antibodies may not reach the level to raise a graft rejection.

Support for these assumptions may be derived from the experiments of Cornish and Scott (1966). They implanted lyophylised homologous and heterologous cardiac valves in sheep. When these tissues were used in myringoplasty no graft rejection was observed, but transplanted into the musculus rectus abdominis, the reaction was vigorous. A comparable observation was done by van den Broek (1968) with incus grafts in rats. When a fresh homologous incus was transplanted into the middle ear no reaction was found. However when transplanted into the musculus tibialis anterior a transplantation reaction was observed.

Summarizing it may be concluded in view of these considerations that with a large number of the afore mentioned tissues a successful healing of persistent perforations in the drum membrane can be obtained. However it must be noted that there are some factors which make it very difficult to predict exactly the successful score which can be obtained with a definite graft.

1. There is a large variability in the surgical techniques used.

2. Many authors fail to report the size and the anatomical position of the perforation as well as the age of the patients. These factors may seriously influence the final results.

3. The follow-up periods are usually rather short. With many techniques the initial successful results appeared to decrease after longer follow-up periods. Therefore a follow-up period of at least several years seems necessary to make final conclusions on the used technique.

4. In many cases no data are reported on the cause of failure. In appreciating the usefulness of a tissue in myringoplasty it is very important to know if the failure is due to a recidive of the otitis or to the behaviour of the graft.

Apart from this and in spite of the lack of satisfactory data on the healing process of the drum membrane and the effect of the graft on this process, several important facts can be distilled from the reported clinical data to obtain successful healing of a persisting perforation in the drum membrane.

1. The middle ear space and the external meatus has to be free from inflammatory processes, when a functional reconstruction is being performed (Zöllner 1955). If this cannot be realized, the inflammatory process had to be

graft (Beickert, 1958) from transsected hair follicles and glands remains present until the graft is completely transformed into tissue of the drum membrane. This chance is much higher if split skin is used, since in this case a much higher number of these skin organs are transsected (Wullstein 1960) In addition to this, split skin may easily necrotize (House & Sheehy 1961 Guilford, 1962 Kraus et al 1964 Bellucci, 1966) and reperforation will occur In view of these considerations it will be clear that the long term results obtained with external body skin grafts were very bad 30–70 / failure (Beickert 1958 Thorburn 1960 1963 House & Sheehy 1961 Guilford, 1962 Mawson & Pickard, 1962 Wright 1963 Örtegren, 1964 Salén, 1964)

Far better results were obtained with pedicled (Frenckner 1955a, b Sooy 1964 Mulcahy & McAfee 1964 Robinson 1966) and free grafts (Plester & Nijsten, 1959 House & Sheehy 1961 Pfaltz et al 1962 Mulcahy & McAfee, 1964 Patterson et al 1967) from the deep part of the meatal skin The development of graft cholesteatoma is very unlikely because this part of the meatal skin does not contain hair follicles or glands Since this tissue is adapted to the temperature and the humidity of this region the absence of inflammatory processes and excessive desquamation can be explained Stenosis of the meatus sometimes occurring as a consequence of the healing of the denuded meatus (Sheehy 1964) is a disadvantage of this method Besides this, it must be noticed that the used meatal skin does not only consist of a layer of epithelium and subepithelial connective tissue, but also of a periosteal layer Therefore it seems likely to assume that the periosteal layer may contribute to the successful results, in view of the favourable results obtained with the use of periost only (Chiossone, 1964 Berger & Hessberg, 1965).

From the excellent results reported with autologous tissues of mesodermal origin like fascia (Heermann 1962 Örtegren 1964 Patterson et al. 1967 Sale 1969), vein (Austin & Shea 1961 Wright, 1963 Tabb & Rutledge, 1964 Austin, 1965) perichondrium (Goodhill et al., 1964) and fat from the ear lobe (Ringenberg, 1962) it seems reasonable to conclude that they are very suitable in myringoplasty The usefulness of some of them (ear-lobe fat, vein and perichondrium) is confined to small perforations, since the obtainable size of pieces of these tissues is not large enough to close large perforations. Moreover vein contains many elastic fibres, so the edges of the graft tend to curl and the contact with the drum is lost (Richards & McGee, 1965) Although initially successful results were obtained in about 90 / of the cases with meatal skin and autologous tissues of mesodermal origin a slight decrease of this percentage was observed after a follow-up period of several years. Wright (1963) and Austin (1965) suggested that in these cases the perforations were closed in highly atrophic membranes. They advised therefore the complete removal of this area. Moreover it appeared that these reperforations occurred in those ears in which a large perforation was present. This might have been an additional cause of the bad results To prevent reperforation in these cases, several surgeons decided to use combined grafts (Kley 1963 Kraus et al., 1964 Guilford, 1962 Mulcahy & McAfee, 1964 Bienias, 1964 Kup, 1967). The results appeared to be successful although reported follow-up periods are in many cases rather short and reperforation usually occurs one or two years post-operatively

During recent years homologous tissues (Schrimpf 1954 amnion Forman 1960 cornea Jansen, 1963 perichondrium Trombetta, 1963 pericard Nickel, 1963 Mitchell, 1967 Bogomilsky 1969 vein Chalat 1964 Marquet, 1968 drum membrane Albrite & Leigh 1966 dura) and even heterologous tissues (Cornish & Scott 1966 1968 cardiac valves Goodhill 1966 gelfoam) have been introduced in myringoplasty With some of those tissues the results seem as successful as with the autologous tissues of mesodermal origin However apart from the method of Chalat (1964), using vital homologous drum membranes, no real homografts have been

CHAPTER II

EXPERIMENTAL STUDY ON THE HEALING PROCESS OF THE DRUM MEMBRANE

A. INTRODUCTION

From the clinical data reported in the first chapter it will be clear that the use of tissue transplantation is essential for obtaining successful healing of a persisting perforation in the drum membrane. Some suggestions have been made on the role played by the graft in the healing process, but without adequate experimental evidence. Moreover fundamental data on the wound healing process of the drum membrane are extremely scarce.

In the past decennium, it is true, several animal studies on the integration of tissues in the drum membrane have been reported. But, except for the investigations of McMinn and Taylor (1966) who studied the early phase of the healing process they only concerned a small number of experiments in which the integration of different tissues was studied after follow-up periods of several months (Tiedemann, 1960 Withers et al., 1963 Richards & McGee, 1965 Paparella, 1967 Rogers & Snow 1968 Salén, 1968). The object of the present study was to examine the healing process of the drum membrane after producing a traumatic perforation and moreover to study the effect of transplanted tissues on this process and the fate of the grafts itself.

In the first part of this investigation, these problems were studied on cats with histological techniques. The second part deals with the same problems studied autoradiographically on the drum membrane of mice. With the latter technique more information could be obtained on the amount and the localization of the mitotic activity during the healing process.

B. HISTOLOGICAL STUDY IN CATS

1 Methods

The experiments were performed on adult cats weighing between two and four kg. The animals were anesthetised with sodium pentobarbital (30 mg/kg body weight) administered intraperitoneally. The surgical procedure was carried out using a Zeiss operating microscope, while surgical sterility was maintained. The drum membrane was reached from a retro-auricular incision after the meatus had been opened a few millimeters from the anulus tympanicus. With a needle a perforation (2–3 mm in diameter) was made in one of the ventral quadrants. In a certain number of ears the drum membrane was completely removed.

The perforations were left open or closed with autologous fat, connective tissue, full thickness skin from the meatus or with gelfoam (imbibed with wound fluid) and subsequently the retro-auricular wound was closed. Each animal received post-operatively 30.000 I U penicilline i.m. (Mycofarm) daily for three days. After survival times varying from one day to thirty weeks post-operatively the animals were sacrificed. The temporal bones were dissected and fixed in Bouin's solution after removal of the bony wall of the middle ear to facilitate fixation of the drum membrane. After 48 hours of fixation, the drum membrane and part of the meatal skin were dissected out after severing the connections between malleus and incus and tensor tympani. Then the specimens were decalcified in Kristensen's fluid, dehydrated in alcohol, embedded in paraffine and serially sectioned

eradicated in such a way that complete healing can be performed with antibiotics within a short time.

2 The tympanic membrane has to be stimulated to proliferate, either by mechanical or by chemical (etching) removal of the scar tissue from the edges of the perforation If the graft is placed on the outer side of the membrane the epithelium that may be covered by the graft has to be removed to avoid the development of cholesteatoma between graft and drum membrane.

3 The graft has to be fixed in such a way that no displacement can occur and the close contact with the drum will persist

4 The graft must show sufficient vitality or structural solidity (in the case of avital tissues or fresh tissues which may lose their vitality after transplantation) to close the perforation until the various layers of the drum membrane had closed the grap.

5 The graft may not develop pathological phenomena as a consequence of the change in environmental conditions, which may seriously interfere with the healing process.

6 The final resorption of the graft is not a necessary demand but important to obtain optimal hearing results.



Fig. 2. Retraction of the epithelial and mucosal layer from the edge of the perforation, 30 minutes after perforating. × 210.

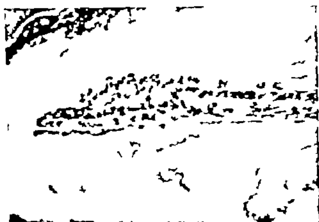


Fig. 3. Part of the edge of the perforation covered with exudate, containing leucocytes, after 24 hours. The epithelium is hypertrophic and the collagenous bundles are fragmented. × 210.

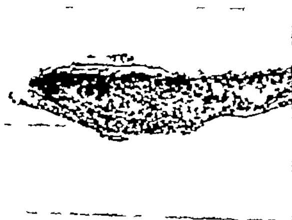


Fig. 4. The highly hyperplastic epithelium already migrating around the edge of the perforation, after 48 hours. × 150.

(7μ) parallel to the long process of the malleus. The sections were stained with hematoxylin eosin

The next series of experiments were performed

1 Small perforations which were left open
2 Total perforations which were left open
3 Small perforations closed with fat.
4 Small perforations closed with connective tissue
5. Small perforations closed with full thickness skin
6 Small perforations closed with gelfoam.
7 Total perforations in which the middle ear and part of the meatus were filled with gel foam

From each series three ears of each survival time were studied

2 *The normal drum membrane*

Although the drum membrane of the cat is smaller and thinner than that in man there are no essential differences in structure. The middle layer of the pars tensa consists of two layers of collagenous bundles. Th fibres in the outer layer show a radial arrangement, those in the inner layer a circular The middle layer of the pars flaccida consists of more loose connective tissue, without special fibre arrangement On the outer side, the membrane is covered with keratinizing epithelium near the anulus and the handle of the malleus consisting of 2 to 3 celllayers and in the other parts of the membrane of one celllayer (Fig. 1) On its inner surface the membrane is lined by the mucous membrane of the tympanic cavity

3 *Small perforations which were left open*

The edge of a fresh perforation fixed within 30 min after perforating is shown in Fig. 2. In an area of about 100–300μ around the perforation the membrane was thickened, caused by swelling of the middle layer The epithelial and mucosal layers were usually retracted from the edge of the perforation.

After 24 hours the oedematous area was further extended. The middle layer was less stainable and often fragmented The epithelial cells showed a marked hypertrophy (Fig. 3) No remarkable changes were observed in the mucosa. During the next days the number of celllayers strongly increased, while keratinization occurred In some regions the epithelium migrated around the edge of the perforation and even below the membrane, making contact with the hypertrophic mucosa (Fig. 4). This situation however does not form the end of the healing process, as was suggested by Dunlap and Schuknecht (1947) These phenomena were most pronounced in the regions of the edge, situated close to the anulus, the handle of the malleus and the plicae mallei If the edge of the perfora



Fig 1 Structure of the normal drum membrane of a cat near the anulus (pars tensa) × 270.

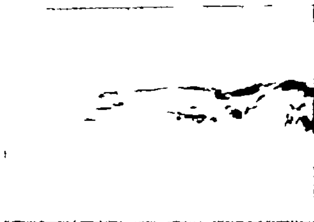


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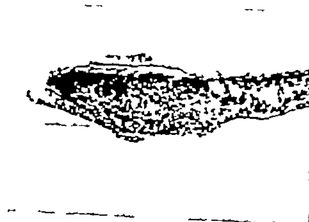


Fig. 4. The highly hyperplastic epithelium already migrating around the edge of the perforation, after 48 hours. × 150.

tion was on a greater distance from these sites, corresponding to a localization in the central part of the pars tensa, these phenomena were much less pronounced (Figs. 5, 6 7) These dif*ferences in activity remained present during the* entire healing process.

From the fourth day on, young mesenchymal cells could be found in the middle layer in the most active regions of the edge of the perforation Thereafter the number of these cells strongly increased, while also small capillaries became visible. In this stage of the healing process the difference in tissue activity in the various areas of the edges was most remarkable At the most active sites, the epithelium sometimes consisted of 15 to 20 celllayers while in these parts of the edge, situated in the centre of the pars tensa only 2 to 3 celllayers could be observed The same phenomenon applied to the amount of mesenchymal cells and the disappearance of the *fibres of the old middle layer (Figs. 5, 6 7) The* epithelium moved across the defect followed by the mesenchymal cells and the mucosa gradually narrowing the gap (Fig. 5). The defect was closed first by the confluence of the epithe*lium and afterwards by the middle layer and the* mucosa From this series of ears most perforations appeared to be closed after 10 to 14 days. In the next days the various tissues of the membrane gradually became thinner and about two weeks after closure, the drum membrane had regained its normal dimensions. Sometimes, epithelial cysts filled with horny lamels and persisting for longer periods, could be found in the healed part of the membrane (Fig. 8). The newly formed middle layer initially consisting *of connective tissue with many fibroblasts* was transformed within 4 to 8 weeks into a thin layer of dense connective tissue with only scattered fibroblasts. This new middle layer however failed to show the characteristic fibre *arrangement.*

From this series all traumatic perforations healed spontaneously except in one case in which the defect was not closed 23 weeks after perforating (Fig. 9) No inflammation or traces of a passed inflammatory process could be found in this case. The edges of the persisting perforation consisted of dense connective tissue, covered with a thin layer of epithelium A stri*king difference in the size of the edge was obser*ved dependent on the distance to anulus, handle of the malleus and plicae mallei The smallest part of the edge, situated in the central part of the pars tensa, consisted only of a small thickened border of dense connective tissue. The biggest part showed a large amount of irregular coursing strands of collagenous fibres in a large area At a short distance from this scar tissue the drum membrane showed its normal appearance (Fig. 10)

4 Total perforations which were left open

Although small perforations of the drum produced traumatically usually heal spontane*ously in animals as well as in man total or* subtotal defects only rarely heal in man Initially these defects tend to heal but complete closure occurs only incidentally For that reason we studied the healing process of the drum mem*brane in cats after complete removal of the mem*brane in ten ears, moreover part of the bony anulus and the adjacent meatal skin was removed in three ears.

No essential differences with the small perforations could be found in the healing process, except for a much more violent course of the process Seven days after removal of the drum a very thick rim of tissue was formed near the anulus. This rim consisted of a thick keratinizing epithelial layer covering the edge of the perfo*ration and a thick layer of highly vascularized* young connective tissue covered by the mucosa (Figs 11a b) The horny lamels were pushed forward in the direction of the regeneration The regenerative activity appeared to be largest in the upper parts of the anulus After two weeks a new membrane was formed on a distance of about 2 mm The epithelium consisting of about 10 to 15 celllayers at the edge of the perforation decreased to 2 to 3 celllayers towards

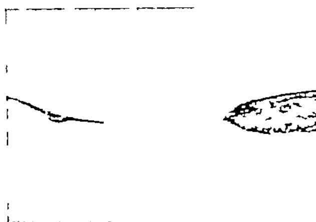


Fig. 5. Two opposite parts of the edge of the perforation after five days, illustrating strong difference in activity in relation to their location. The left part is situated in the centre of the pars tensa, the right one near the plica mallei × 35.

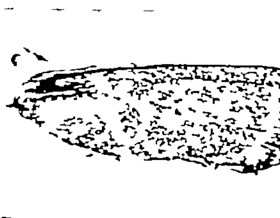


Fig. 6. Higher magnification of the right edge of Fig. 5, showing large amount of epithelial and mesenchymal cells 90.



Fig. 7. Higher magnification of the left edge of Fig. 5, showing the migration of thin sheet of epithelial cells across the perforation. Remnants of the original middle layer are still present, while no new fibroblasts can be observed 150.



Fig 8. Epithelial cyst filled with a horny [illegible] in the healed area, after 9 weeks × 90.



Fig 9 Two opposite parts of a traumatically produced perforation which failed to heal completely after 23 weeks. Note the large difference in size of the two parts × 30.



Fig 10. Higher magnification of the right edge of the perforation of Fig. 9. The edge consists of a big amount of strands of dense connective tissue, covered by one layer of flat epithelial cells × 80.





Fig. 11. Parts of the regenerating drum membrane, one week after total perforation. a. In the region of the pars flaccida and b. in the region of the pars tensa × 50.

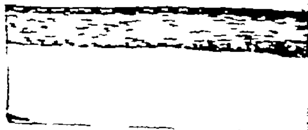


Fig. 12. Structure of the regenerated membrane, 5 weeks after total perforation × 210.

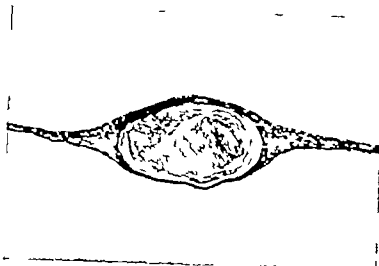


Fig 8. Epithelial cyst filled with a horny mass in the healed area, after 9 weeks × 90.



Fig 9. Two opposite parts of a traumatically produced perforation which failed to heal completely after 23 weeks. Note the large difference in size of the two parts × 30.



Fig 10 Higher magnification of the right edge of the perforation of Fig. 9. The edge consists of a big amount of strands of dense connective tissue, covered by one layer of flat epithelial cells × 80.

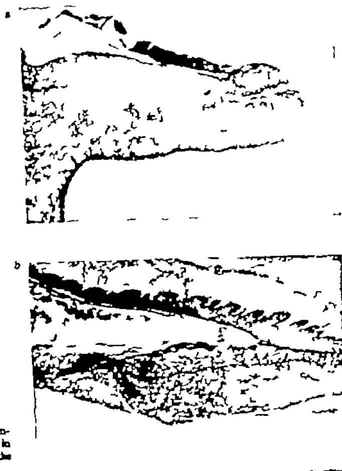


Fig. 1. Parts of the regenerating drum membrane, one week after total perforation a, in the region of the pars flaccida and b, in the region of the pars tensa × 50.

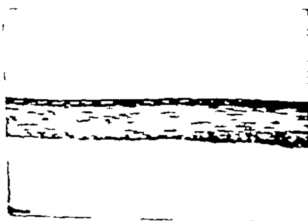


Fig. 12. Structure of the regenerated membrane, 3 weeks after total perforation × 110.

the anulus. A comparable decrease in thickness could be observed in the connective tissue Five weeks after removal of the membrane the defect appeared to be healed even in those ears in which part of the bony anulus had been removed The new membrane was nearly completely flat From the malleus, only the processus brevis and adjacent structures situated in the plane through the anulus were incorporated while the handle protruded free into the middle ear as was also recently reported by McIntire and Benitez (1970) The new membrane appeared to be composed of a thick middle layer consisting of connective tissue with many fibroblasts covered by two layers of epithelial cells and a one celllayer thick mucosa (Fig. 12) Locally accumulations of capricious coursing fibres were found (Fig. 13). In the next months the middle layer was transformed into a thin dense connective tissue layer with only scattered fibroblasts (Fig. 14)

In one ear a large epithelial cyst filled with keratine was observed on the handle of the malleus 20 weeks after perforating (Fig. 15) An open communication with the external meatus appeared to exist, as was concluded from the serial sections. Presumably this cyst originated from an incomplete removal of the epithelium from the malleus.

5 *Small perforations closed with fat*

In these ears small perforations were closed with subcutaneous fat, obtained from the retro-auricular wound

Twenty four hours after perforating the membrane showed an extensive oedema while a slight hyperplasia of the epithelium was observed near the edge of the perforation Generally the oedema was much more extensive in those ears in which the perforations were closed with a graft than in those which were left open The graft appeared to be more or less infiltrated by poly morphonuclear leucocytes, presumably already present in the tissue when it was dissected from the retro-auricular wound made 20 minutes earlier Leucocytes were also observed in the middle layer of the drum near the perforation. After 2 days the hyperplasia of the epithelium was strongly increased mainly in those regions where the edge of the perforation was situated close to the anulus, the plicae mallei and the processus longus The epithelial cells had been pushed up as it were against the graft resulting in a splitting into two layers one moving along the outer side of the graft and one along the medial side of the graft (Figs. 16a, b) After 4 days the migrating epithelium already covered a large part of the graft, while mesenchymal cells from the middle layer of the drum migrated into the graft. Simultaneously the mucosal layer started to migrate along the medial side of the graft. After one week the amount of mesenchymal cells in the graft was strongly increased, while also small capillaries were observed (Fig. 17) Immediately below the epithelial and mucosal layer a condensation of young fibroblasts was formed. Within two weeks the whole graft was covered with a thick epithelial layer on the outer side and a hypertrophic mucosal layer on its medial side. Underneath these epithelial linings a thick strand of vascularized connective tissue was formed. The short strands of epithelial cells, initially covering part of the medial side of the graft became surrounded by connective tissue at this stage and gradually disappeared within four to six weeks, while sometimes horny masses could be observed (Figs. 18 19) In the same time the epithelium and the mucosa gradually returned to their normal appearance. The fat grafts, revealing areas with lymphocytes and phagocytotic cells, appeared to be considerably decreased in size and were partly replaced by connective tissue. However a large individual difference in the condition of the grafts was observed. Sometimes nearly the whole graft was transformed into a flat disc of connective tissue, infiltrated by lymphocytes and foam cells (Fig. 20) In other cases a large quantity of fatty tissue surrounded by a layer of subepithelial and submucosal connective tissue was still present. These differences may—

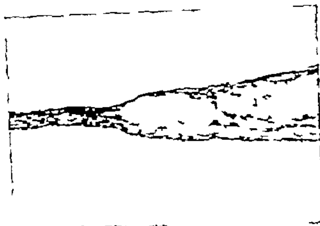


Fig. 13. Part of the new membrane, showing a local thickening of the middle layer, 5 weeks after total perforation. × 150.

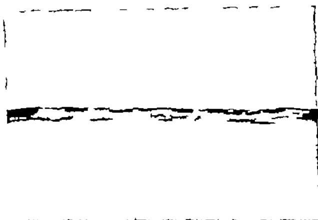


Fig. 14. Structure of the central part of the new membrane, 12 weeks after total perforation. 340.

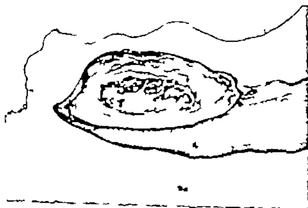


Fig. 15. Survey of the new membrane, developed 20 weeks after total perforation, showing the handle of the malleus (bearing large epithelial cyst) protruding free into the middle ear cavity. The membrane appeared to be completely flat at time of dissection. The folding appearance in this micrograph is due to the histological procedure. 35.


a


b

Fig 16. Survey (a) × 35 and higher magnification (b) × 90 of a perforation closed with a fat graft, after 2 days. The hyperplastic epithelium is already migrating along the graft. The original middle layer of the membrane is disappearing.



Fig 17 Survey of a perforation, one week after closing with a f t graft The infiltration of the graft by mesenchymal cells from the middle layer of the membrane is clearly visible



Fig. 18. Part of the healed drum membrane, 4 weeks after fat graft. The graft shows numerous lymphocytes, islands of degenerating epithelium and strands of connective tissue 35.

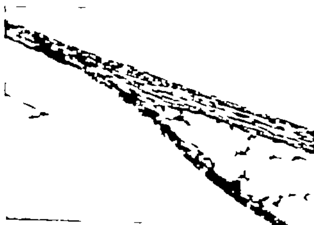


Fig. 19. The healed drum membrane, 6 weeks after fat graft. The graft is surrounded by subepithelial and submucosal layer of connective tissue 150.



Fig. 20. Survey of the integrated fat graft after 6 weeks. The fat tissue is nearly completely replaced by connective tissue 35.


a


b

Fig 16. Survey (a) × 35 and higher magnification (b) × 90 of a perforation closed with a fat graft, after 2 days. The hyperplastic epithelium is already migrating along the graft. The original middle layer of the membrane is disappearing.



Fig 17 Survey of a perforation, one week afte closing with a fat graft. *The infiltration of th graft by mesenchymal cells from the middl layer of the membrane is clearly visible* × 35



Fig. 8. Part of the healed drum membrane, 4 weeks after fat graft. The graft shows numerous lymphocytes, islands of degenerating epithelium and strands of connective tissue × 33.

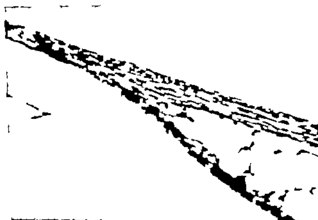


Fig. 19. The healed drum membrane, 6 weeks after fat graft. The graft is surrounded by subepithelial and submucosal layer of connective tissue × 150.



Fig. 20. Survey of the integrated fat graft after 6 weeks. The fat tissue is nearly completely replaced by connective tissue × 33.

at least partly due to the amount of fat which was transplanted In nearly all cases only small remnants of the graft could be found after five months. The remnants, consisting of fat cells, foam cells and some lymphocytes, were surrounded by a layer of dense connective tissue covered with an epithelial and mucosal layer which had its normal appearance (Fig. 21)

6 Small perforations closed with connective tissue

In these experiments subcutaneous connective tissue which was dissected from the retroauricular wound was used As in the fat grafts numerous polymorphonuclear leucocytes were observed in the transplanted connective tissue after twenty four hours. The course of the healing process showed a great resemblance to that in which fat was used The epithelium moved along the outer side of the graft and initially also at a short distance along the medial side, while occasionally epithelial cells in the graft were observed After about five days a massive migration of mesenchymal cells into the graft was found (Fig. 22)

Already a few days after transplantation the connective tissue revealed signs of degeneration This was concluded from the presence of pycnotic nuclei and a decrease in the stainability of the tissue After two weeks the main part of the graft appeared to be replaced by newly formed connective tissue containing numerous capillaries and lymphocytes. In this way as it were, a new cutaneous layer underneath the already closed hyperplastic layer was formed. At that time only poor stainable remnants of the connective tissue were present (Fig. 23) In the next weeks the epithelial layer became gradually thinner while close below the epithelium and mucosa a layer of dense connective tissue was formed, enclosing remnants of the graft and giant cells (Fig. 24) After five months the original position of the perforation could only be recognized by a slightly thickened middle layer containing rather many fibroblasts.

7 Small perforations closed with full thickness skin

The skin grafts were dissected from the canal wall about 3 mm outside the drum and placed over the perforation No retaining pack was used, because the graft adhered sufficiently to the drum membrane. The situation one week after transplantation is illustrated in Fig. 25. The meatal skin appeared to be composed of a very thick layer of dense connective tissue (dermis) containing some glands and hair follicles, covered with keratinized stratified squamous epithelium. After one week a thick epithelial layer moving up against the grafts, was observed at the transition of graft and drum membrane (Fig. 26). Sometimes this epithelium had already made contact with the epithelium of the skin. In the transitional zone mesenchymal cells and also small capillaries were already present in the graft The epithelium of the drum, at the edges of the perforation covered by the graft was hyperplastic.

After two weeks the epithelium of the drum was everywhere continuous with the graft epithelium In this stage the mucosal layer separated from the graft by a small layer of young connective tissue had already closed the gap. That part of the epithelium of the drum covered by the graft had lost its continuity and only small islands of swollen cells were found. The disappearance of this epithelium showed a striking resemblance with the one infiltrated in the fat and connective tissue grafts. After two weeks the amount of mesenchymal cells originating from the middle layer of the drum membrane was strongly increased in the graft replacing the original tissue of the dermis The residual part of the latter tissue was less stainable and contained pycnotic nuclei Moreover a large quantity of lymphocytes, especially close below the epithelium was observed

Four to six weeks after perforating, the hyperplasia of the epithelium was strongly decreased especially in the transitional zone. The main part of the original dermis had been replaced by new

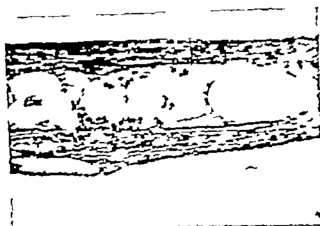


Fig. 21. Part of a fat graft after 5 months, showing residual fat cells and foam cells × 150.



Fig. 22. Section through the edge of the perforation closed with connective tissue after 5 days, illustrating the migration of epithelium along the graft and the infiltration of epithelial and mesenchymal cells into the connective tissue × 90.



Fig. 23. Survey of the healing membrane, weeks after connective tissue graft. The transplanted tissue is almost entirely replaced by highly vascularised connective tissue with many fibroblasts. → remnant of the graft × 90.

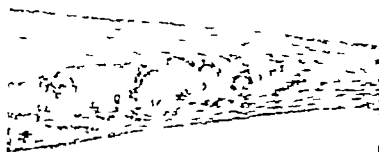


Fig 24. Giant cells between the submucosal and subepithelial connective tissue layer 8 weeks after a connective tissue graft × 110.



Fig 25 Section through a meatal skin graft after 1 week. In this section the epithelium of the drum membrane had already made contact with the graft epithelium. The epithelium of the drum, partly covered by the skin graft, is strongly hypertrophic × 35.



Fig 26 Higher magnification of the transitional area between drum membrane and skin graft of Fig. 25 × 90.

connective tissue containing many fibroblasts while often a clear continuity between the middle layer of the membrane and the new dermis of the graft could be observed (Figs. 27 28, 29). Moreover a remarkable decrease in the number of hair follicles and glands was found. After two to three months no remnants of the original dermis were found. In this stage of the healing process part of the epithelium of the skin graft was quite similar to that of the drum membrane (Fig. 30). After six months the graft appeared to be strongly flattened by a decrease in size of the connective tissue layer

8. Small perforations closed with gelfoam

Besides the use of autologous tissues, we also studied the effect of gelfoam implants on the healing pattern of the drum membrane. In these experiments the traumatic perforations were closed with a small piece of sterile gelfoam imbibed with wound fluid.

After one day the same phenomena could be observed as with the use of autologous tissues. The gelfoam was infiltrated by a large amount of polymorphonuclear leucocytes, presumably arising from the wound fluid. A strong hyperplasia as well as a capricious infiltration of epithelium into the graft was observed after two days (Fig. 31), becoming much more extensive in the next days. This phenomenon was much more pronounced than in the cases of fat or connective tissue grafts, probably due to the spongy character of the gelfoam. After five days a great number of mesenchymal cells and capillaries were present in the middle layer of the membrane, but only a slight infiltration of these elements into the gelfoam was observed. After one week the epithelium, and also the mucosal layer had nearly closed the gap. The epithelial cells, both on the meatal surface and in the gelfoam, showed a pale and swollen appearance (Fig. 32). After two weeks a strong increase of the number of mesenchymal cells was found, while also small capillaries were present (Fig. 33). In this stage, the graft was covered with a thick keratinizing layer of epithelium, while islands of degenerating epithelial cells were present in the graft.

After four to five weeks no remnants of the gelfoam implant were observed. Immediately below the epithelial and mucosal layer a small strip of fibrous connective tissue was present. Between these layers a great number of lymphocytes, keratine and connective tissue strands were found (Fig. 34). In the following weeks the membrane became gradually thinner The epithelium and the mucosa showed their normal appearance after two months, while the middle layer was still rather thick, consisting of connective tissue with many fibroblasts (Fig. 35).

After five months this layer appeared to be transformed into dense connective tissue with only scattered fibroblasts. Occasionally local



Fig 27 Section through meatal skin graft after 4 weeks 35.

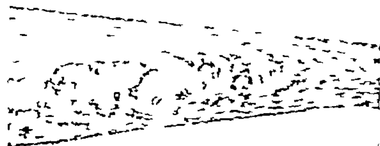


Fig 24. Giant cells between the submucosal and subepithelial connective tissue layer 8 weeks after a connective tissue graft × 110.



Fig 25. Section through a meatal skin graft after 1 week. In this section the epithelium of the drum membrane had already made contact with the graft epithelium. The epithelium of the drum, partly covered by the skin graft, is strongly hypertrophic × 35.



Fig 26. Higher magnification of the transitional area between drum membrane and skin graft of Fig. 25 × 90.

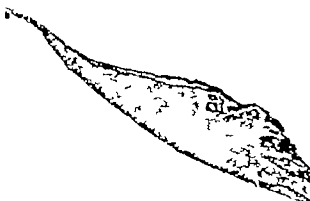


Fig. 30. Section through a meatal skin graft after 9 weeks. In this graft the original dense connective tissue of the dermis is completely replaced by connective tissue with many fibroblasts originating from the drum membrane × 35.



Fig. 31. Capricious infiltration of a gelfoam graft by epithelial cells from the hyperplastic edge of the perforation, after 2 days × 90.



Fig. 32. Section through a gelfoam graft after one week, illustrating the large amount of swollen epithelial cells infiltrated into the graft × 90.

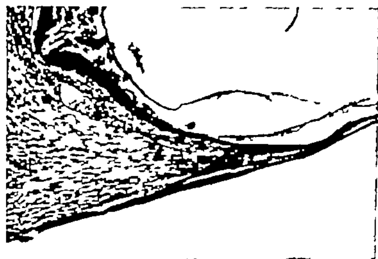
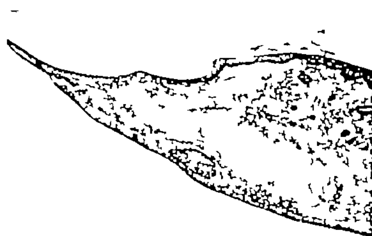


Fig 28. Higher magnification of the skin graft of Fig. 27 illustrating the continuity between the middle layer of the drum membrane and the newly formed connective tissue of the skin graft × 90.

a



b

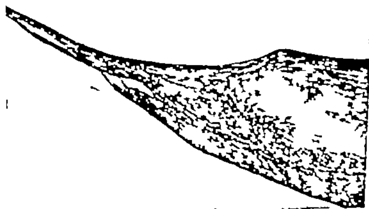


Fig 29. Survey (a) × 45 and higher magnification (b) 90 of a skin graft after 6 weeks. The thickness of the epitheli m of the skin graft is already strongly decreased In the dermal portion of the graft remnants of the original connective tissue are still present. Close to the mucosal layer a small sland of degenerating epithelial cells is isible.

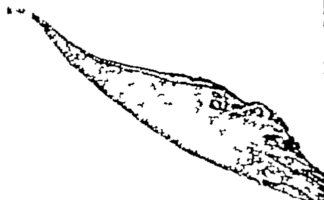


Fig 30. Section through a meatal skin graft after 9 weeks. In this graft the original dense connective tissue of the dermis is completely replaced by connective tissue with many fibroblasts originating from the drum membrane 35.



Fig 3 Capricious infiltration of gelfoam graft by epithelial cells from the hyperplastic edge of the perforation, after 2 days 90.

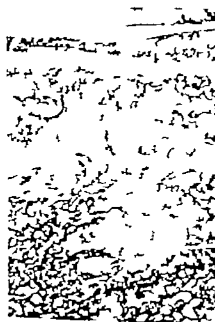


Fig 3 Section through a gelfoam graft after one week, illustrating the large amount of swollen epithelial cells infiltrated into the graft 90.



Fig 33 Section through a gelfoam graft after 2 weeks. The graft is covered with a thick layer of epithelium and contains a large amount of mesenchymal cells × 150.



Fig 34 Part of the healed drum membrane four weeks after a gelfoam graft The graft is replaced by mesenchymal cells, lymphocytes and islands consisting of a horny substance × 90.



Fig 35 Part of the healed membrane 7 weeks after a gelfoam graft. The middle layer is still rather thick and contains many fibroblasts × 150.

thickenings, built up of collagenous fibres were observed.

9. *Total perforations closed with gelfoam*

In six ears the drum membrane had completely been removed and the middle ear cleft and part of the meatus had been filled up with gelfoam.

Within five weeks a new membrane appeared to have developed. In contrast with the total perforations which were left, the handle of the malleus was incorporated in the new membrane, although the membrane lacked the original conical shape. In most cases tissue bridges mainly composed of connective tissue lined with mucosa appeared to exist between the membrane and the medial wall of the middle ear (Fig. 36). In this stage of the healing process the epithelium consisted of several celllayers. The middle layer was very thick and appeared to consist of loose connective tissue except for the region immediately below the epithelium which contained a large amount of fibres (Fig. 36). In the following weeks the membrane gradually became thinner. The middle layer was transformed into a fibre rich connective tissue (Fig. 37).

After five months the thickness of the membrane was further decreased, although the middle layer revealing a large number of irregularities, was still rather rich in fibroblasts (Fig. 38). As in the other experiments, no signs of the characteristic fibre distribution in the normal membrane could be observed.

C AUTORADIOGRAPHICAL STUDY IN MICE

In the preceding study on the drum membrane of the cat a clear microscopic picture was obtained of the course of the healing process of a traumatic perforation and the effect of a tissue transplant on this process, in addition to its ultimate fate. However in view of the technique used no clear data could be obtained on the magnitude and the localization of the mitotic activity in the normal membrane and during the healing process. Therefore we decided to perform a series of identical experiments with the use of an autoradiographical technique as had been used by McMinn and Taylor (1966) and Litton (1968). In this way the mitotic activity could be determined at every wanted date, using radioactive thymidine (H -thymidine). This nucleoside is specifically incorporated into the chromosomal DNA in the S-phase of the mitotic cycle during which the amount of DNA is reduplicated (Quastler & Sherman, 1959). H thymidine disappears from the circulation within one hour after i.p. injection (Hughes et al., 1958) by destruction or excretion (von Potter et al., 1958). In this time all cells which are in the S-phase will incorporate this substance into their DNA. In our experiments we used a dose of 1µC/g body weight and an exposure-time of one hour. In view of this low dose and short exposition time, toxic effects and radiation damage to the cells may be excluded according to the experiments of Johnson and Cronkite (1959), Cronkite et al. (1961) and Fry and Lesher (1961). They all observed damaging effects only at much higher doses and longer exposition times.

This study was performed on the drum membrane of mice. Apart from the high amount of H -thymidine which would have been required if cats had been used, the drum membrane in mice can easily be surveyed and perforated without incising the meatus. In this way a possible influence of a wound in the meatus on the mitotic activity of the drum membrane could be excluded, although our experiments on cats did not give rise to such an influence.

1 *Methods*

The experiments were performed on Swiss mice (b.w. 18–25 g) anesthetized by means of an intraperitoneal injection of pernocton. With the use of a small glass funnel (diameter 1.5 mm) placed into the meatus, the drum membrane could be surveyed. Small perforations (0.5–1 mm diameter) were made with a glass needle. The perforations were left open or closed with a small



Fig 36. Section through the regenerated membrane partly connected with the medial wall of the middle ear 5 weeks after total perforation and filling up of the middle ear and part of the meatus with gelfoam. The middle layer of the membrane is very thick and consists of a subepithelial layer with many fibres and of a loose highly vascularised tissue towards the wall of the middle ear × 35.

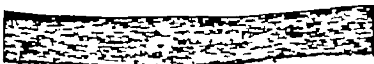


Fig 37 Part of the new drum membrane, 12 weeks after total perforation and filling up of the middle ear and part of the meatus with gelfoam × 210



Fig 38. Local thickening, consisting of dense connective tissue in the regenerated drum membrane, 20 weeks after total perforation and filling up with gelfoam × 150

piece of subcutaneous fat from the retro-auricular region. The surgical procedure was performed with sterilized instruments and with the use of an operation microscope.

After follow-up periods ranging from 18 hours to 3 weeks, 1μC H^3-thymidine (s.a. 1 9 C/mM Radiochemical Centre, Amersham, Engl.) per gram body weight was administered intraperitoneally. All animals were injected at 10.00 a.m. to avoid variations in the mitotic activity due to the diurnal rhythm and they were killed by decapitation one hour after injection of the isotope.

The whole petrous bone was dissected and after removal of part of the bony wall of the middle ear fixed for six hours in a solution containing 5 formaldehyde and 5 / trichloracetic acid, at 0 C. In this way the specimina were simultaneously fixed and decalcified without loss of nucleic acid content. After dehydration in graded alcohols the tissues were embedded in paraffine and sectioned serially (7μ). The sections were mounted on gelatinized slides and coated with Ilford G5 nuclear emulsion using the dipping technique (Kopriwa & Leblond, 1962). After an exposure time of 14 days the autoradiographs were developed, fixed, stained with methylgreen-pyronine and mounted in DPX. From each survival time four membranes were studied.

In order to obtain a survey of the localization of the labelled cells, the drum membrane was reconstructed with the use of the autoradiographs. For this reconstruction the sections 1 9 17 etc of the serially sectioned membrane were studied using the anulus and the handle of the malleus as fixed points. Each section was reproduced as a straight line, divided into segments, corresponding with 150μ of the membrane. The number of labelled cells was indicated at the base of each segment using different symbols for the epithelium, connective tissue and mucosa. In some cases the number of labelled cell could not be indicated in one diagram, because of the large amount of labelled cells in the various tissues. In these cases separate diagrams of the epithelium and connective tissue were made. Moreover from each drum membrane some characteristic sections were drawn, demonstrating the exact position of the labelled cells. The straight lines in the diagrams refer to the position of these sections. In addition the course of the thickness of the epithelial layer around the perforation during the healing process was visualized in a graph in order to compare this with the localization of the mitotic activity. For these graphs only sections coursing through the perforations have been used. The first section cuts the edges of the perforation near the handle of the malleus, the last one near the anulus. On the ordinate the thickness of the epithelial layer is indicated in μ above the zero line the thickness on the upper side of the perforation and below this line that underneath the perforation. The position of the lines, indicated by the arabic figures agrees with that in the diagram of the drum membrane.

The vascular pattern in the normal membrane and during the healing process was visualized by means of intracardial perfusion of the anesthetized animal with Indian ink. After completed injection the temporal bones were removed, fixed in formaldehyde and cleared in methyl benzoate after dehydration in alcohol.

2 *Morphology of the normal drum membrane*

A schematic drawing of the drum membrane of the mouse, including the fibre distribution (visualized with the polarization microscope) is shown in Fig. 39. Apart from the large surface of the pars flaccida, relative to the pars tensa, there are no important differences between the structure of the drum membrane of mice and that in men and cats. The long axis of the membrane measures about 3.5 mm and the short axis about 2.5 mm, while the minimal thickness of the pars tensa is only 3 4μ. The neck of the malleus is shell-shaped and its lateral edge incorporated in the membrane (Fig. 40) and perpendicular to the handle of the malleus has a fibrous connection with the bony anulus. The outer



Fig. 36. Section through the regenerated membrane partly connected with the medial wall of the middle ear 5 weeks after total perforation and filling up of the middle ear and part of the meatus with gelfoam. The middle layer of the membrane is very thick and consists of a subepithelial layer with many fibres and of a loose highly vascularised tissue towards the wall of the middle ear. × 35.

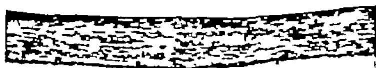


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Fig. 38. Local thickening, consisting of dense connective tissue in the regenerated drum membrane 20 weeks after total perforation and filling up with gelfoam. × 150.

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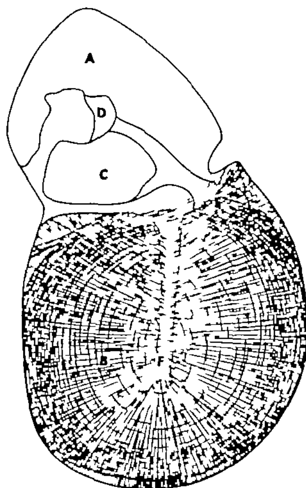


Fig 39 Schematic drawing of the drum membrane with fibre distribution of a mouse. A = pars flaccida, B = pars tensa, C = neck of the malleus, D = incudo-malleolar joint, E = processus brevis, F = handle of the malleus.

side of the membrane is covered with keratinizing squamous epithelium consisting of one celllayer except for the areas near the anulus and the handle of the malleus, where two celllayers can be observed (Fig. 41). Just as in other mammals the middle layer of the pars tensa is composed of an outer layer with a radial and an inner layer with a circular fibre arrangement, while the pars flaccida reveals no definite fibre distribution. At the inner side the membrane is covered with one layer of flat cells continuous with the epithelial lining (mucosa) of the middle ear In the vascular system three regions can be distinguished a marginal net near the anulus, a central net near the neck and the handle of the malleus and a vascular net in the pars flaccida (Fig. 42)

3 Mitotic activity in the normal drum membrane

The mitotic activity in the structures of the normal drum membrane in mice is shown in Fig. R1[1]). The localization of the labelled cells in the pars tensa appeared to be confined to a small area near the anulus and the handle of the malleus. In the pars flaccida however the labelling was scattered diffusely over the whole membrane. The ratio between the number of labelled epithelial cells, fibroblasts and mucosal cells was in the pars tensa 35 5 1 and 15 5 1 in the pars flaccida, the total number of labelled cells in both parts being about equal (n = 3) Since the surface area of the pars tensa is about twice that of the pars flaccida the mitotic activity in the structures of the pars flaccida is two times higher than in the pars tensa.

In Fig. R2 the mitotic activity in a drum membrane obtained from a mouse with a purulent middle ear disease is shown Compared to the normal membrane, the mitotic activity was extremely enhanced not only near the anulus, the handle of the malleus and the pars flaccida but even in the central part of the pars tensa. The same activity but to a lesser extent is applied to the connective tissue and the mucosa A micrograph of this ear (Fig. 43) shows the middle ear cleft filled with inflammatory cells. The outer surface of the membrane showed a thick keratinizing squamous epithelium while the dense connective tissue of the middle layer was transformed into a highly vascularized layer of connective tissue with a large amount of fibroblasts.

4 Small perforations which were left open

a. Pars tensa

About eighteen hours after perforating the pars tensa, the membrane, including the pars flaccida, was oedematous. Both epithelium and mucosa, which were slightly hypertrophic, were retracted from the edges of the perforation but to a larger degree than was observed in the drum membrane of the cat. In the membrane as well as in the middle ear cleft polymorphonuclear leucocytes were found

[1]) Appendix



Fig. 40. Section trough the drum membrane of a mouse, parallel to the handle of the malleus, showing the lateral edge of the neck of the malleus incorporated into the membrane on the dividing line between pars tensa and pars flaccida. ×35.



Fig. 41. Higher magnification of the drum membrane (pars tensa) with annulus of a mouse. ×110.



Fig. 42. The vascular pattern in the untreated drum membrane of a mouse.

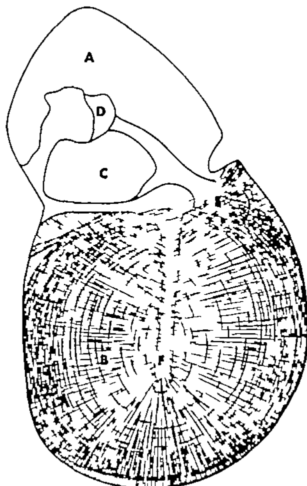


Fig 39 Schematic drawing of the drum membrane with fibre distribution of a mouse. A = pars flaccida, B = pars tensa, C = neck of the malleus, D = incudo-malleolar joint, E = processus brevis, F = handle of the malleus.

side of the membrane is covered with keratinizing squamous epithelium consisting of one celllayer except for the areas near the anulus and the handle of the malleus, where two celllayers can be observed (Fig. 41) Just as in other mammals the middle layer of the pars tensa is composed of an outer layer with a radial and an inner layer with a circular fibre arrangement while the pars flaccida reveals no definite fibre distribution At the inner side the membrane is covered with one layer of flat cells continuous with the epithelial lining (mucosa) of the middle ear In the vascular system three regions can be distinguished a marginal net near the anulus, a central net near the neck and the handle of the malleus and a vascular net in the pars flaccida (Fig. 42).

3 Mitotic activity in the normal drum membrane

The mitotic activity in the structures of the normal drum membrane in mice is shown in Fig. R1[1]) The localization of the labelled cells in the pars tensa appeared to be confined to a small area near the anulus and the handle of the malleus. In the pars flaccida however the labelling was scattered diffusely over the whole membrane. The ratio between the number of labelled epithelial cells, fibroblasts and mucosal cells was in the pars tensa 35 5 1 and 15 5 1 in the pars flaccida, the total number of labelled cells in both parts being about equal (n = 3) Since the surface area of the pars tensa is about twice that of the pars flaccida the mitotic activity in the structures of the pars flaccida is two times higher than in the pars tensa.

In Fig. R2 the mitotic activity in a drum membrane obtained from a mouse with a purulent middle ear disease is shown Compared to the normal membrane, the mitotic activity was extremely enhanced not only near the anulus, the handle of the malleus and the pars flaccida, but even in the central part of the pars tensa. The same activity but to a lesser extent, is applied to the connective tissue and the mucosa A micrograph of this ear (Fig. 43) shows the middle ear cleft filled with inflammatory cells. The outer surface of the membrane showed a thick keratinizing squamous epithelium, while the dense connective tissue of the middle layer was transformed into a highly vascularized layer of connective tissue with a large amount of fibroblasts.

4 Small perforations which were left open

a. Pars tensa

About eighteen hours after perforating the pars tensa the membrane, including the pars flaccida was oedematous. Both epithelium and mucosa, which were slightly hypertrophic, were retracted from the edges of the perforation, but to a larger degree than was observed in the drum membrane of the cat. In the membrane as well as in the middle ear cleft polymorphonuclear leucocytes were found

[1]) Appendix



Fig. 40. Section trough the drum membrane of mouse, parallel to the handle of the malleus, showing the lateral edge of the neck of the malleus incorporated into the membrane on the dividing line between pars tensa and pars flaccida × 35.



Fig. 4 Higher magnification of the drum membrane (pars tensa) with anulus of mouse × 210.



Fig. 42. The vascular pattern in the untreated drum membrane of mouse.



Fig 43 Section through the middle ear of a mouse with an inflammatory middle ear disease. The middle ear cavity contains many inflammatory cells. The epithelium of the drum membrane is stratified, while the thin layer of dense connective tissue of the middle layer is transformed into highly vascularised connective tissue with many fibroblasts × 90.

The number of labelled cells eighteen hours after perforating is shown in Fig. R3 The position of the retracted epithelium is indicated by the dotted line The retraction was less pronounced near the handle of the malleus than in the other areas Presumably this may originate from a firmer adherence of the epithelium to the middle layer in this region. Compared with the normal membrane no significant increase in the number of labelled cells was observed at that time.

After two days the amount of epithelial cells was strongly increased at the edge of the perforation especially in those areas where the edge was close to the malleus and the anulus (Fig. R4) as was also observed in the cat. Moreover the number of labelled epithelial cells was strongly enhanced near the anulus and the malleus and even in the pars flaccida (Fig R5) The same applied to the localization and the number of labelled mucosal and connective tissue cells, except for a remarkable high labelling of the mucosa on the handle of the malleus (Fig. R5 R6) The hyperplasia of the epithelium appeared to have been further increased after three days. Near the anulus, a slight hyperplasia of the mucosa was observed as well as the presence of mesenchymal cells. That part of the original middle layer denuded of epithelium and mucosa showed an avital appearance in this stage. A further increase in the amount of labelled cells was observed except for the pars flaccida (Fig. R7).

In the following days the hyperplasia of the epithelium further increased, while a clear migration towards the perforation was found, gradually narrowing the gap The hyperplasia was most remarkable in those parts of the edge situated close to the anulus and the handle of the malleus, as is illustrated in Fig. R8, while sometimes accumulations of dead epithelial cells were observed near the handle (Fig. R9) In this stage the mucosa was highly hyperplastic and was already moving towards the perforation. The diagrams in Fig. R9 and R10 not only show a further increase in the number of labelled cells, but also an enlargement of the area of mitotic activity towards the perforation

Only precisely at the outer edges where the epithelial cells were piled up no labelling was observed A further increase was also observed in the amount of labelled mesenchymal cells, mainly restricted to the anulus and the handle of the malleus The original avital lamina propria became gradually replaced by a layer of young connective tissue cells with some capillaries on the medial side covered with the hyperplastic mucosa The vascular pattern of the membrane, six days after perforation is shown in Fig 44. From a limited area of the vascular net in the anulus and the handle of the malleus new vessels appeared to have developed towards the healing perforation

After ten days most perforations were closed The mitotic activity decreased rapidly after



Fig. 44. The vascular pattern of the drum membrane, 6 days after producing a traumatic perforation (p) in the pars tensa.

closure of the epithelial layer. A few days later also the middle layer and the mucosa appeared to be closed. In the following days the epithelium became gradually thinner and two to three weeks after perforating the drum membrane had regained its original structure (Fig. R11). In the region where the perforation was made, only a few labelled cells were present. Except for a mucosal fold between drum and processus brevis and a small irregularity in the middle layer no essential differences with the normal membrane were found.

b. Pars flaccida

In view of the differences in structure and vascularization between pars tensa and pars flaccida, we studied the healing process of a traumatically produced perforation in the pars flaccida. Eighteen hours after perforating both epithelium and mucosa were retracted from the edge of the perforation, but to a much lower extent than in the pars tensa. The epithelium was hypertrophic and on the edge and in the middle ear cleft extravasated erythrocytes and also polymorphonuclear leucocytes were observed. The whole pars flaccida and the adjacent part of the meatal skin in which the pars flaccida gradually continues, showed a severe oedema. In some cases already a slight increase of the number of labelled cells was found in the pars flaccida, while a remarkable increase in labelling was observed in the anulus of the pars tensa, returning to its normal level after about three days (Fig. R12).

After three days the highly hyperplastic epithelium appeared to have migrated around the edge of the perforation. In this stage a large amount of labelled epithelial cells was present in the pars flaccida, in the adjacent part of the meatal skin and in the region of the handle of the malleus. In contrast to our findings after perforating the pars tensa, the localization of the labelled cells was not confined to distinct areas, but scattered diffusely on the whole surface. The mucosal lining of the pars flaccida, the ossicular chain and the wall of the middle ear as well as the submucosal connective tissue showed many labelled cells (Fig. R13).

In the following days the epithelium, followed by the connective tissue and the mucosal layer migrated towards the center of the perforation, closing the perforation in a concentrical way. The vascular pattern six days after perforating (Fig. 45) was quite different from that observed in the pars tensa. A characteristic pattern of vessels radiating towards the perforation had developed. Most perforations appeared to be healed after ten days and afterwards the pars flaccida regained its normal anatomical structure.

5 Small perforations closed with fat

On the analogy of our experiments on the cat, some fresh traumatically produced perforations in mice were closed with an autologous fat graft. Eighteen hours after transplantation no significant change in the mitotic activity was found. The retraction of the epithelium and mucosa was much less than if the perforations were left open. This may have been caused by the adherence of

the fat to these tissues. After two days the epithelium was already pushed up against the fat graft as was observed in the experiments on the cats (Figs. 46 47) The increase in the number of labelled cells appeared to be confined to the same areas as when the perforation was left open (Fig. R14) In the following days the epithelium migrated as a thick layer along the outer side of the graft, while the graft became infiltrated with mesenchymal cells from the middle layer of the membrane as was found in the cat Besides scattered capillaries and islands of fibroblasts in the graft, a clear concentration of these elements was found immediately below the epithelium nearly covering the whole transplanted tissue, and the mucosa (Fig. 48). In this stage a large amount of labelled cells was observed in the basal layer of the epithelium migrating along the graft, as well as in the graft itself (Fig. 49) After ten days nearly all perforations appeared to be closed The graft was covered by a thick layer of keratinizing epithelium. Between epithelium and the fat graft infiltrated by lymphocytes and fibroblasts, a thick layer of highly vascularized connective tissue was found (Fig. 50) On its medial surface the graft was covered by the mucosa. The mitotic activity had strongly decreased after closure of the perforation as is illustrated in Fig. R15. Afterwards the epithelium as well as the connective tissue layer became gradually thinner while the disappearance of the fat occurred just as we observed in the cat.



Fig 5 The vascular pattern of the drum membrane, 6 days after producing a traumatic perforation (p) in the pars flaccida.

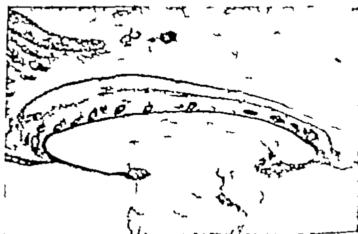


Fig 46 Section through the membrane, 2 days after closing a perforation with a fat graft × 35.



Fig 47 Higher magnification of the membrane of Fig. 46 showing the migration of epithelial cells along the fat graft × 90



Fig 48. Section through a fat graft after 7 days. The migrating epithelium has nearly closed the gap. The graft is infiltrated by capillaries and fibroblasts, especially concentrated close below the migrating epithelium × 90.



Fig 49 Autoradiograph of the healing membrane, 7 days after fat graft, showing labelled cells in the basal layer of the epithelium, migrating along the graft. In the graft numerous labelled mesenchymal cells can be observed 90

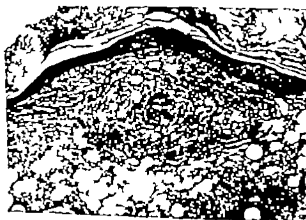


Fig 50. Section through fat graft after 0 days. Below the epithelium which has already closed the gap, large amount of vascularized connectiv tissue is present. Many lymphocytes are observed in the fat tissue × 90.

CHAPTER III

DISCUSSION AND CONCLUSIONS

From the results described it appears that a perforation produced traumatically in the drum membrane of the cat and mouse, just as in man usually heals spontaneously It seems likely to assume that a traumatically perforated normal drum membrane has a much better healing power than the membrane in which a perforation persists after healing of a past chronic otitis. This may be concluded from the fact that in the latter cases, the membrane rarely heals completely after removal of the tissue (scar tissue) at the edges of the perforation In this way a nearly comparable situation may be assumed to be created as in a drum membrane containing a fresh traumatically produced perforation. It has been proved that covering of the perforation with a piece of tissue, as discussed in chapter I is necessary to obtain complete healing. Later on in this discussion we will pay more attention to the function of the graft in the healing process

In our experiments only one case of a traumatically produced perforation in the drum membrane of a cat failed to heal after about six months (Figs. 9 and 10) This lack of healing is difficult to explain because no inflammation or traces of past inflammatory processes could be observed. The microscopical picture of the edges of the perforation, however agrees with that of a persisting perforation in the human membrane. The edge is avascular and consists of a large amount of scar tissue. From these pictures the necessary removal of this tissue, postulated in chapter I as a step in obtaining healing of the perforation will be clear

From the results obtained in the present experimental study the healing process of a traumatically perforated drum membrane may be characterized in the following way Although the drum membrane may be considered as part of the skin, the healing process is different. Bullough & Laurence (1959, 1960) have shown that wound healing of the external body skin occurs by the development of granulation tissue in the wound and an enhancement of the mitotic activity in the epithelium in a circular area around the wound. In the pars tensa of the drum membrane the healing process appeared to occur primarily by migration of epithelial cells from definite proliferative centers situated in or nearby the highly vascularized areas of the membrane, viz. the anulus, the handle of the malleus and the plicae mallei In this respect our experiments could not confirm the assumption of McMinn and Taylor (1966) that at first granulation tissue would be formed and secondly epithelial migration would take place

The migration of the epithelium appeared to occur in a nearly flat plane towards the center of the perforation Secondarily young mesenchymal cells became visible at a short distance from the outer edge of the migrating epithelium The magnitude of both epithelial and mesenchymal cell reaction in various parts of the edge of the perforation was highly dependant on the distance of the edge to the aforementioned vascular areas. (Figs. 5-7) This difference in activity in the various parts of the edge could also be observed in the perforation which did not heal completely It seems likely to assume that the epithelium in those parts of the edge which contain a large number of mesenchymal cells and also capillaries, will proliferate although this could not be confirmed definitely in the cat The formation of epithelial cells in the highly vascularized

areas will certainly contribute to a great extent in closing the perforation, considering the pictures we often observed in those parts of the edge situated in the centre of the pars tensa (Fig. 7). In these areas a large layer consisting only of epithelial cells appeared to migrate across the gap. In mice the main proliferative activity was initially confined to the anulus and the handle of the malleus after perforating the pars tensa. Afterwards this area simultaneously with the extension of the vascular system was enlarged towards the perforation (Figs. R3–R10, 44).

In this manner a traumatic perforation was first closed by the confluence of the epithelium and shortly afterwards followed by the layer of connective tissue cells and the mucosa. After closure, the enhanced mitotic activity decreased rapidly and the membrane gradually regained its original structure (Fig. R11), although no traces of the original fibre distribution (circular and radial) were observed.

The healing process of the drum membrane after a total perforation did not differ fundamentally from that of a small traumatic perforation, except for a much more violent reaction (Figs. 11a, b). This may be due to the traumatization of the highly vascularized areas of the drum. In this way the large gap can be closed. Since in all those ears a flat membrane was formed without the incorporation of the handle of the malleus, we came to the conclusion that the healing process was directed in a nearly flat plane through the anulus. Additional evidence for this conclusion might be derived from the micrograph in Fig. 11b, in which the horny lamels appeared to be pushed forward in this plane. From our experiments we could not conclude which mechanism underlies this directional growing pattern. However an inducing role of the anulus in the regeneration of the membrane as supposed by Goodhill (1966) seems unlikely since a new membrane was even formed if the main part of the bony anulus had been removed.

The large irregularities (Fig. 13) observed in these new membranes may presumably be ascribed to the more violent course of the healing process compared to the cases in which small perforations were made.

In contrast to the total perforations which were left, the handle of the malleus appeared to be incorporated into the new drum, however lacking the original conical shape, if the perforation was filled up with gelfoam. This may be caused by the infiltration of the gelfoam by fibroblasts and capillaries. In this way the handle of the malleus became surrounded by connective tissue which was continuous with the subepithelial connective tissue of the newly formed drum. The origin of the tissue bridges between the new membrane and the medial wall of the middle ear can be thought to develop in a similar way (Fig. 36).

The course of the healing process of a perforation which was closed with a graft appeared to be quite different from that of a perforation which was left open. After covering the defect with a graft, the migration of the epithelium in a flat plane was now blocked and the epithelial cells migrated mainly along the outer side of the graft (Figs. 16, 22, 25, 31 46, 47 R14). The small amount of epithelial cells which initially migrated along the medial side and into the graft disappeared after a short time. Afterwards the transplanted tissue was infiltrated by mesenchymal cells and capillaries and a condensation of these elements was formed immediately below the migrating epithelium and mucosa (Figs. 23, 48, 50). In this way part of the epithelium migrating along the graft had the opportunity to proliferate as could be definitevely proved in our experiments on the drum membrane of mice with the use of H^3-thymidine (Fig. 49). In this stage the bridging of the gap was not only dependent on the supply from distant proliferative areas.

After closure of the perforation reorganization occured. The new middle layer arose from the invaded fibroblasts, especially from the connective tissue which was formed below the epithelial and mucosal layer while the transplanted tissue was reabsorbed. No essential differences in the healing process were observed, if fat, connective tissue, full thickness skin or gelfoam were used, although there were differences in degree. The

CHAPTER III

DISCUSSION AND CONCLUSIONS

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migrate along the graft, which is simultaneously invaded by mesenchymal cells, while capillaries arise. In this way the migrating epithelium obtains a new well vascularized substratum, enabling the epithelium to proliferate (Fig. 49). Although our observations do not give rise to the existence of an effect of a humoral factor from the transplanted tissues on the healing process, this possibility cannot be completely rejected.

In view of these observations it seems likely to assume that a persisting perforation in a partly atrophic drum membrane with scar tissue as a consequence of a past chronic middle ear disease will not heal completely after removal of the scar tissue alone, since the membrane had lost part of its normal healing potency. In these cases successful closure can usually be obtained if the perforation is moreover covered with a graft. According to our observations, the transplanted tissue, becoming vascularized and infiltrated by mesenchymal cells, affords the epithelium which migrates along the graft, the opportunity to proliferate. By this the further course of the healing process is not only dependent on the proliferative activity which may be affected by the chronic disease, in the areas distant from the perforation. Moreover part of the tissue infiltrated into the graft contributes to the formation of the new middle layer. In this way the successful healing of persisting perforations, obtained with the use of various fresh and denatured grafts from different origin, satisfying the conditions presented in chapter I (p. 10), can at least partly be explained.

ACKNOWLEDGEMENTS

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speed of resorption is presumably highly dependent on the structure and the degree of loss of vitality during the first stage of the healing process, before revascularization of the graft has been established.

If gelfoam was used a more capricious and faster infiltration by epithelial cells was observed (Figs. 31 32) These phenomena as well as the rapid absorption are probably due to the spongy character of this substance

We will further go into the behaviour of meatal skin In contrast to the use of external body skin, no pathological phenomena were observed with deep meatal skin, as had been reported by Salén (1968) in a comparable experiment. This may be explained by the fact that the meatal skin is adapted to the climatological conditions in that part of the body We observed a gradual replacement of the dermal portion of the graft by connective tissue arising from the drum membrane (Figs. 28–30) while simultaneously a decrease in the number of hair follicles and gland structures was found In this respect our results disagree with the conclusions made by Salén (1968) after follow up periods of 14 and 25 weeks, that the meatal skin grafts usually integrate with retained viability size and character This difference may be at least partly due to the choice of the observation periods and the amount of transplanted tissue

On the fate of the original epithelium of the graft however we could not decide whether it was replaced by the epithelium of the drum or transformed into it. In view of the observations of McLoughlin (1961) showing a determination of the properties of epithelium by the subepithelial connective tissue, the epithelium of the graft may be transformed into the epithelium of the drum membrane, since the original dermal portion was replaced by connective tissue from the drum

Concerning the reaction of the two parts of the membrane (pars tensa and pars flaccida) a clear difference in the course of the healing process could be observed. In contrast to the healing process in the pars tensa, perforations made in the pars flaccida heal in a concentrical way (Figs. R13 45) This healing pattern showed a great resemblance to that of a defect in the skin, probably due to a greater similarity in structure and vascularization of skin and pars flaccida than of skin and pars tensa. The structure of the pars tensa is highly specialized to the function of sound perception The epithelium of the pars flaccida is because of its structure able to proliferate equally on its whole surface (Fig. 42). In the pars tensa we only observed this in pathological conditions (Fig. R2) The micrograph of the drum membrane of a mouse with an inflammatory middle ear disease (Fig. 43) revealed a transformation of the avascular lamina propria consisting of dense connective tissue into a highly vascularized loose connective tissue with many fibroblasts. In view of these observations, we are not able to conclude whether the cells in the central part of the pars tensa had lost their mitotic activity or whether they cannot divide because of the unfavourable nutritional conditions.

Summarizing the following conclusions may be drawn from this study The wound healing process of the pars tensa of the drum membrane is markedly different from that of external body skin while this process in the pars flaccida shows a great similarity with that of the skin.

The proliferative activity of the epithelium in the normal membrane and also in the first stage of the healing process is mainly confined to the areas with the largest vascular density viz. the anulus (also reported by Litton, 1968), the handle of the malleus, the plicae mallei and the pars flaccida The same applies to the origin of the fibroblasts and the mucosal cells, although the mucosal lining of the ossicular chain and the wall of the middle ear show a large mitotic activity during the healing process (Figs. R5 R10).

The healing of a traumatically produced perforation occurs first by the migration of epithelium from the aforementioned proliferative areas towards the perforation and afterwards also from certain regions nearby the perforation If a perforation is closed with a graft, the direction of the migration is changed and the epithelial cells

APPENDIX

Drum membranes reconstructed from autoradiographs

SUMMARY

The spontaneous healing of a perforation in the tympanic membrane depends on the way it is caused and on the size of the defect. Small perforations which appear as a consequence of a trauma or result from an acute otitis media usually close themselves rather rapidly However larger perforations and perforations arising as a consequence of a chronic middle ear inflammation only rarely heal spontaneously

At this moment a number of surgical techniques are available which can be used in most cases to close these defects of the tympanic membrane These procedures have in common that always a graft is used However a big gap exists in our knowledge of the fundamental aspects involved in the pattern of the wound healing of the tympanic membrane as well as of the role played in it by the graft.

The first part of this monograph contains a critical analysis of the methods used until now to close a persisting perforation of the drum membrane In this chapter it is attempted, on the basis of data from the literature, to select a number of conditions which apparently have to be fulfilled if reconstructive surgery of the tympanic membrane is likely to offer a reasonable chance of success.

In the second part (Chapter II) the results of an experimental study on the tympanic membrane in animals are presented These experiments were carried out on the tympanic membrane of the cat and the mouse In the cat common histological techniques were used in comparing the spontaneous healing process of the perforated tympanic membrane with the effect of using various types of grafts in closing the perforation.

Similar experiments were performed on mice with the use of an autoradiographical technique. Thanks to this latter technique the site and the extent of the mitotic activity of the healing process could be determined in the entire membrane at any given point of time.

From the results obtained it was concluded that the healing process of a perforation in the pars tensa strongly depends on the mitotic activity in fixed parts of the membrane the anulus, the plicae mallei and the handle of the malleus. Also under normal conditions the largest mitotic activity is located in these areas. The migration of the epithelial cells always runs approximately in a flat plane across the perforation The behaviour of the pars flaccida is different from that of the pars tensa both under normal and under traumatic circumstances.

When a perforation in the pars tensa is covered with a graft the migration pattern of the epithelial cells is disturbed. The epithelium is pushed up against the graft and can proliferate because the graft becomes vascularized.

In view of these findings the successful repair of a perforation in the drum membrane with a great number of fresh or denatured tissues may be explained

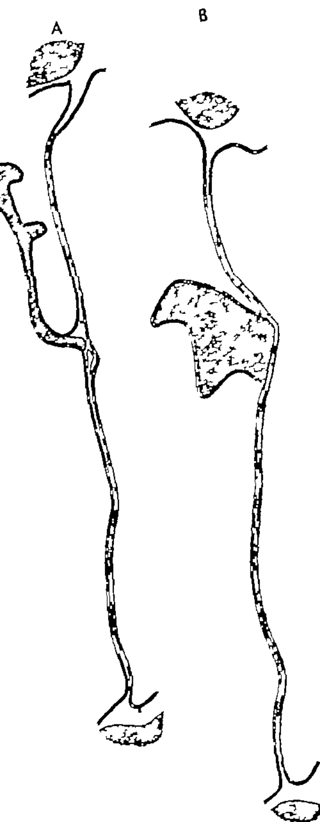
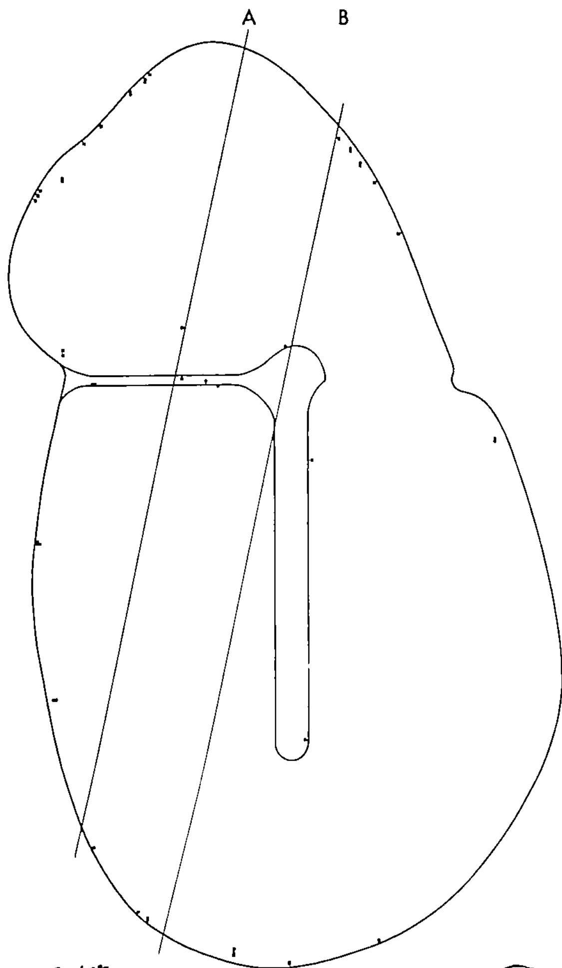


Fig. R1 Reconstruction of the normal drum membrane and some characteristic sections, illustrating the amount and distribution of labelled cells after the administration of H Thymidine. The symbols used in these figures and those to follow have the following meaning:

- = labelled epithelial cell
- □ = labelled fibroblast
- ✓ = labelled mucosal cell
- = borderline of the retracted epithelium.


A
B

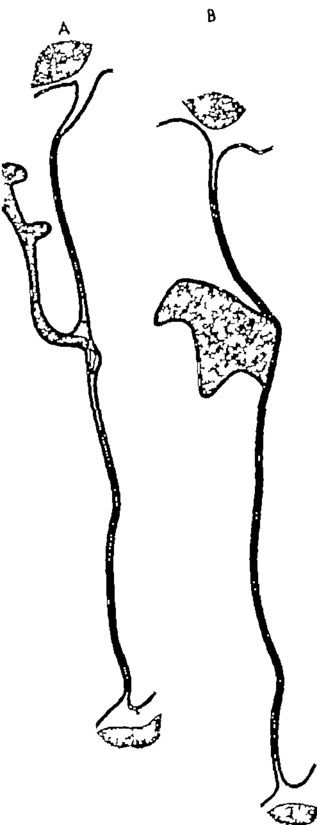
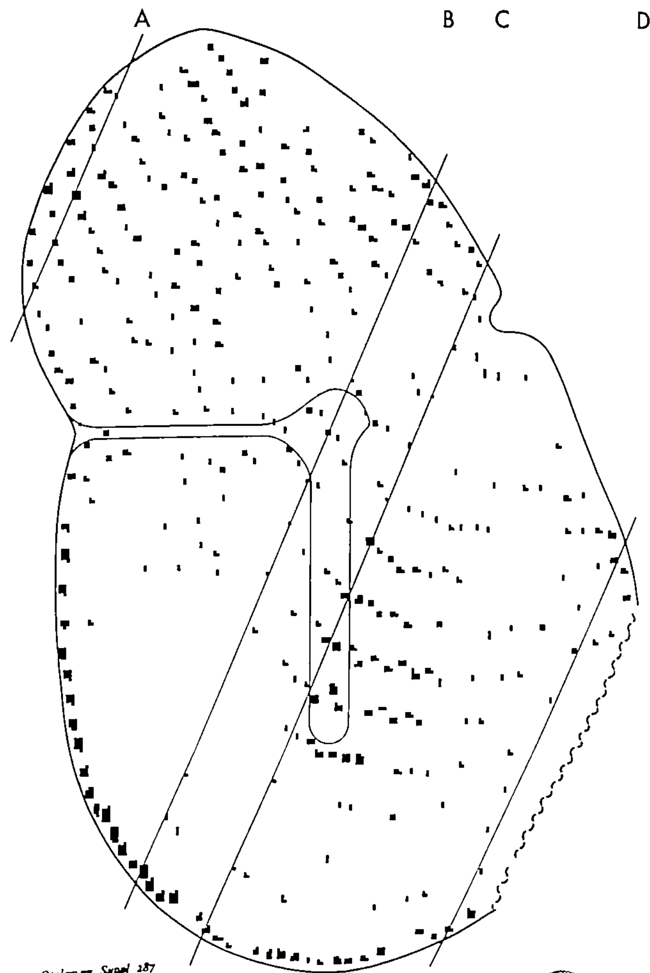


Fig. 81. Reconstruction of the normal drum membrane and some characteristic sections, illustrating the amount and distribution of labelled cells after the administration of H Thymidine. The symbols used in these figures and those to follow have the following meaning

■ = labelled epithelial cell
□ = labelled fibroblast
× = labelled mucosal cell
= borderline of the retracted epithelium.


A
B
C
D

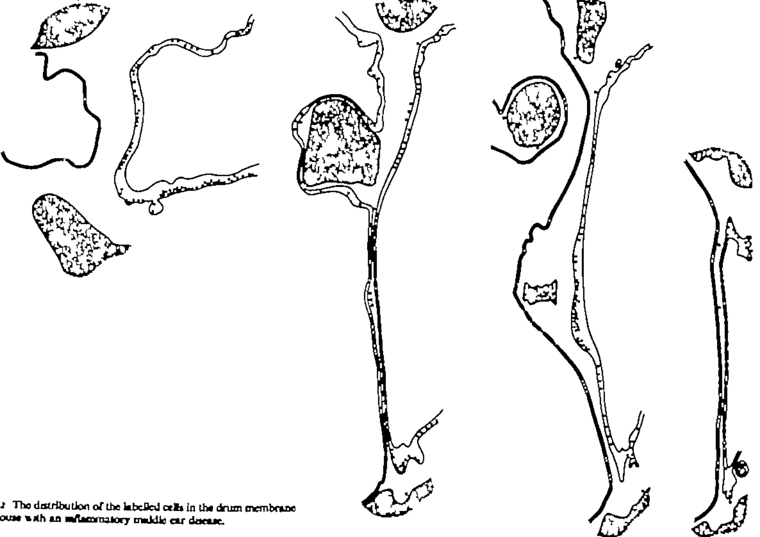
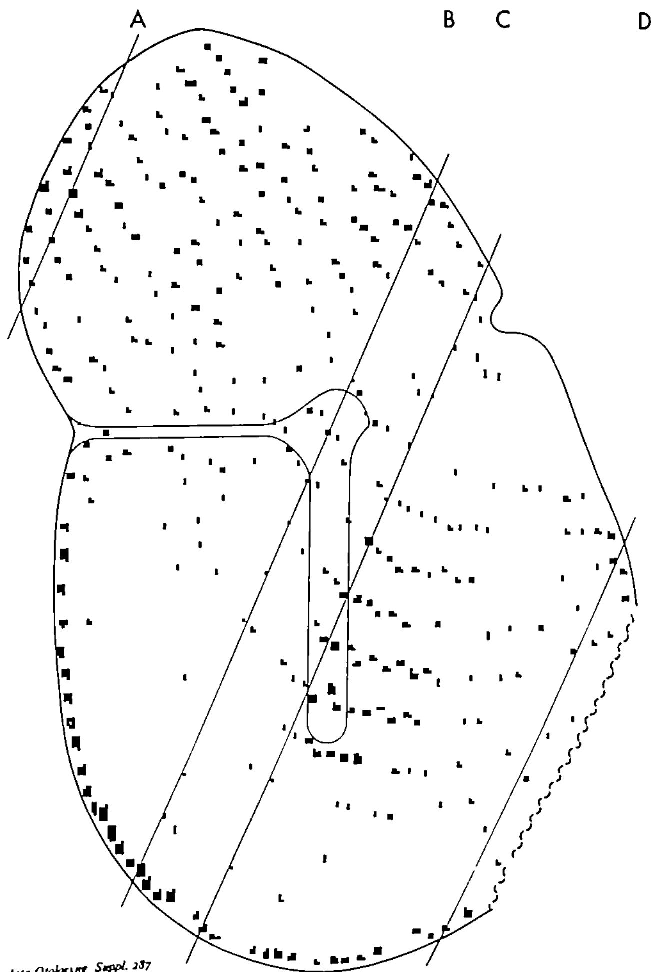


Fig. 8.2 The distribution of the labelled cells in the drum membrane of mouse with an inflammatory middle ear disease.


A
B
C
D

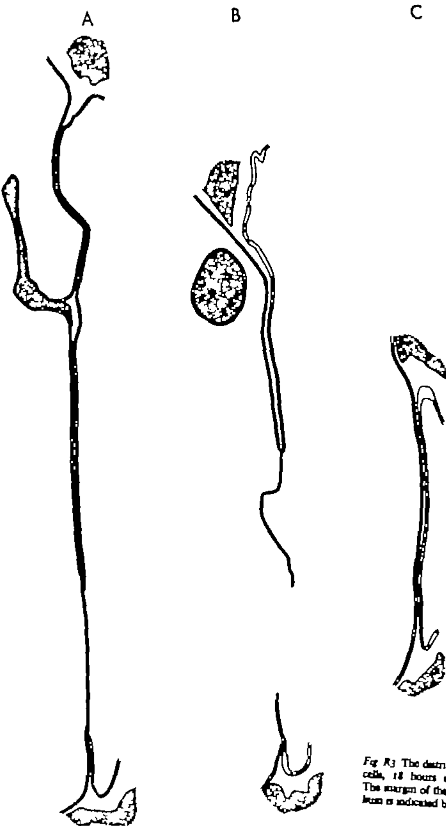
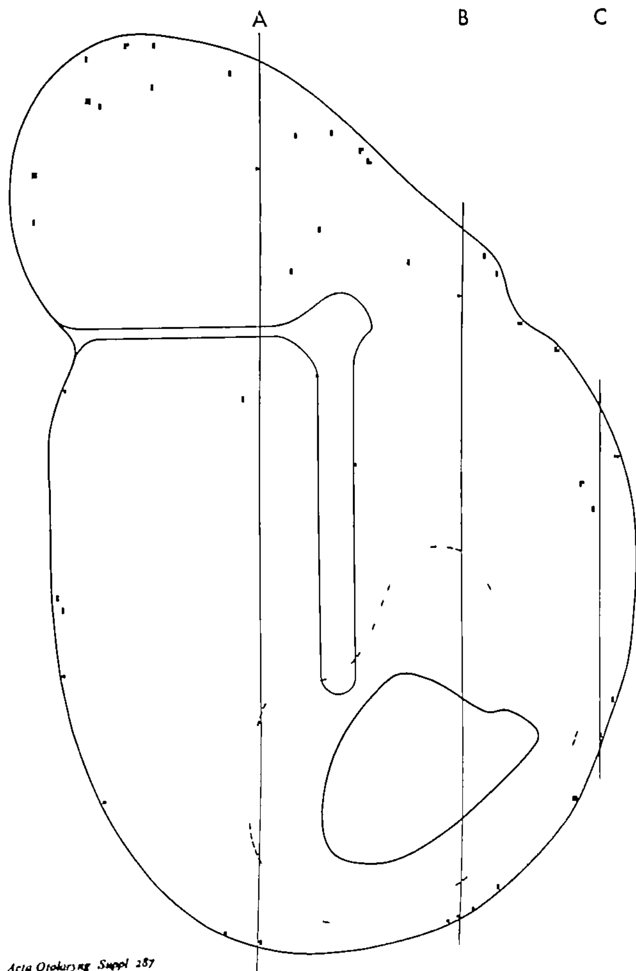


Fig. R3 The distribution of labelled cells, 18 hours after perforating. The margin of the retracted epithelium is indicated by the dotted line.


A
B
C

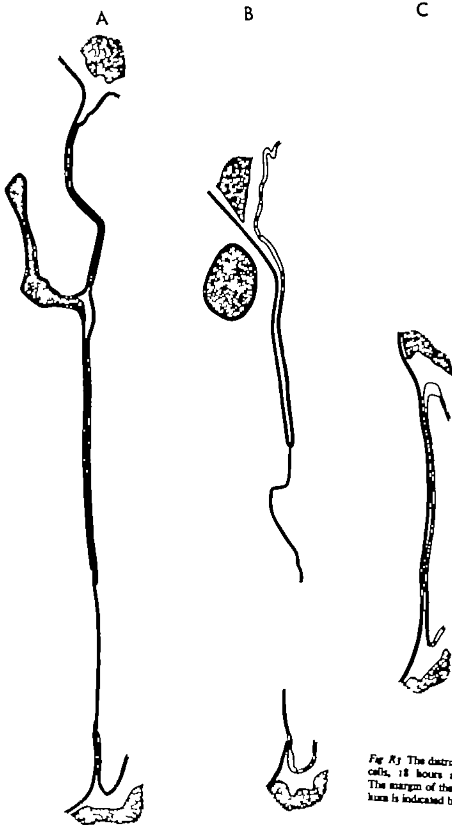


Fig. R3 The distribution of labelled cells, 18 hours after perforating. The margin of the retracted epithelium is indicated by the dotted line.

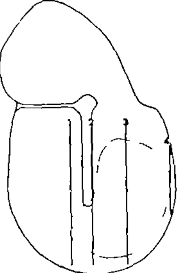


Fig R4 The course of the thickness of the epithelial layer from the handle of the malleus (H) towards the anulus (A) at various distances from the edge of the perforation after 2 days. The vertical lines in the graph, indicated by the arabic figures, correspond to those in the diagram of the membrane (same membrane as in Fig R5). The ordinate indicates the thickness in μ, the upper traces that on the upper side and the lower that underneath the perforation.

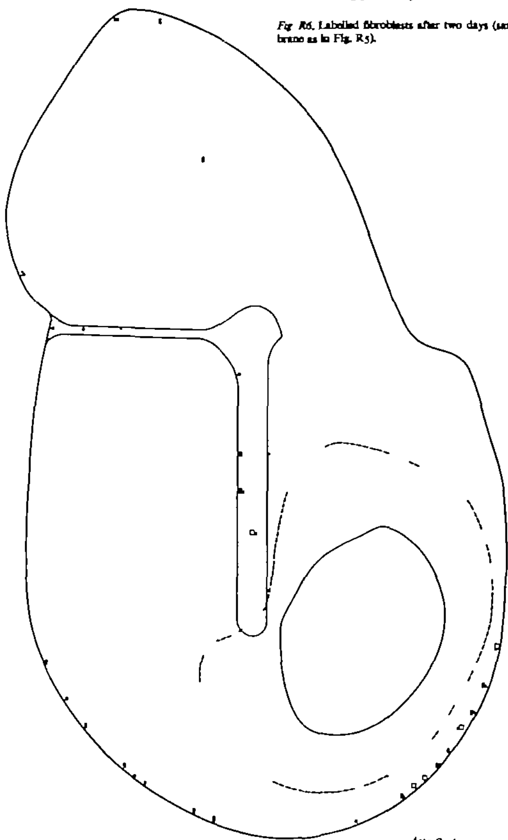


Fig. R6. Labelled fibroblasts after two days (same membrane as in Fig. R5).

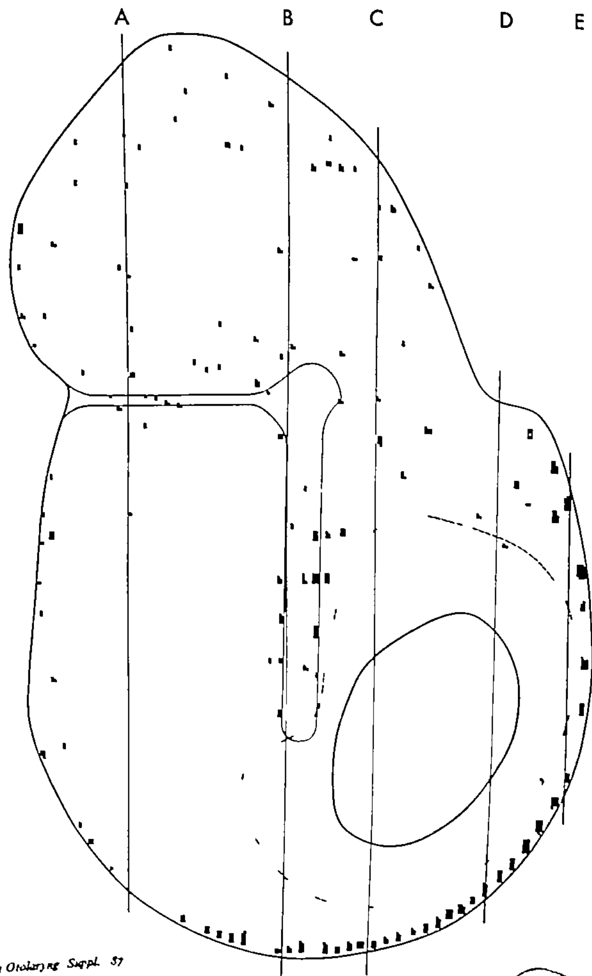
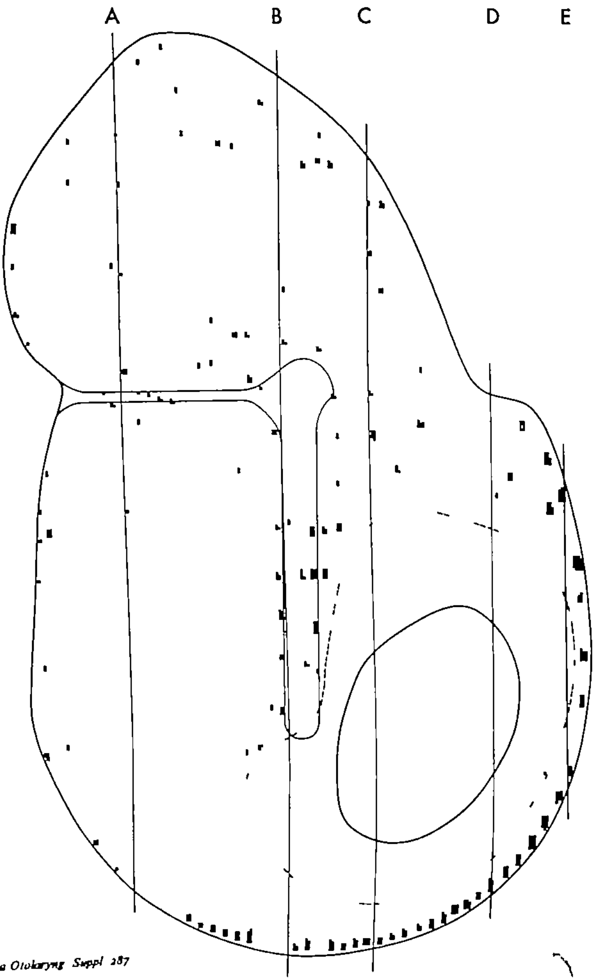

A
B
C
D
E



Fig. R5. Labelled cells after two days.


A
B
C
D
E

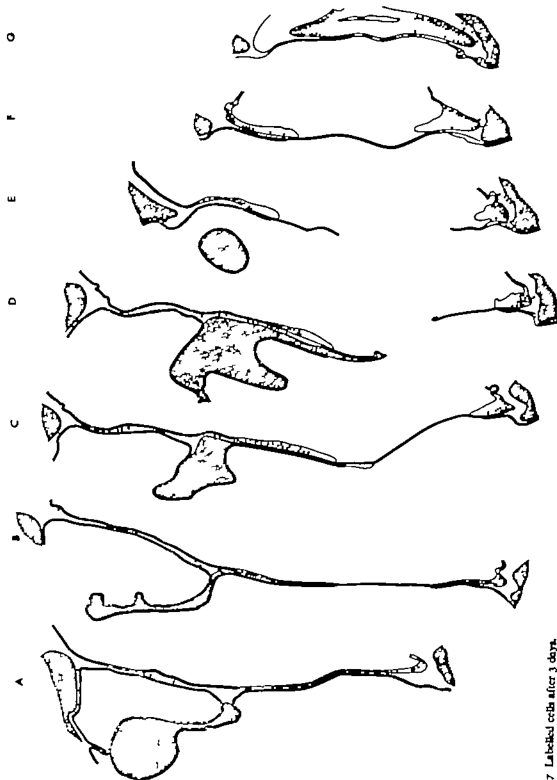
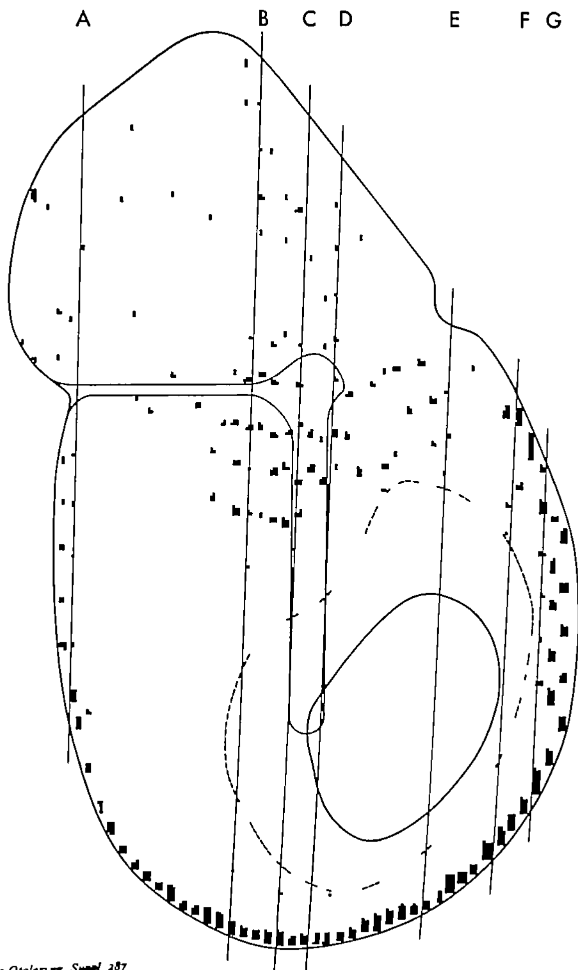


Fig. 87 Labelled cells after 3 days.


A
B
C
D
E
F
G

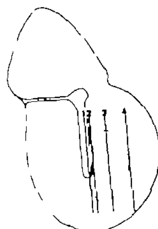
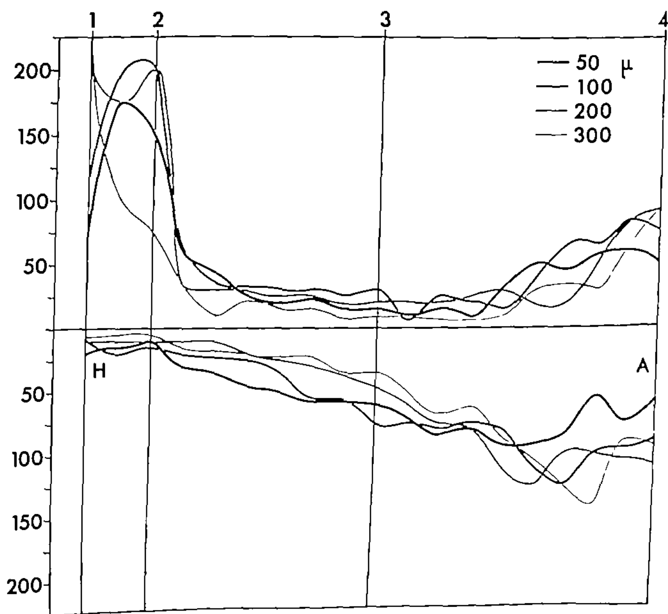


Fig. R8. The course of the thickness of the epithelial layer from the handle of the malleus (H) towards the anulus (A) at various distances from the edge of the perforation after 4 days (same membrane as in Fig. R9). The ordinate indicates the thickness in μ, the upper traces that on the upper side and the lower that underneath the perforation.


1
2
3
4
200
150
100
50
50
100
150
200
50 μ
100
200
300
H
A

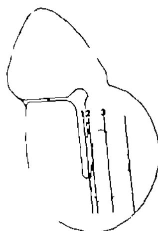
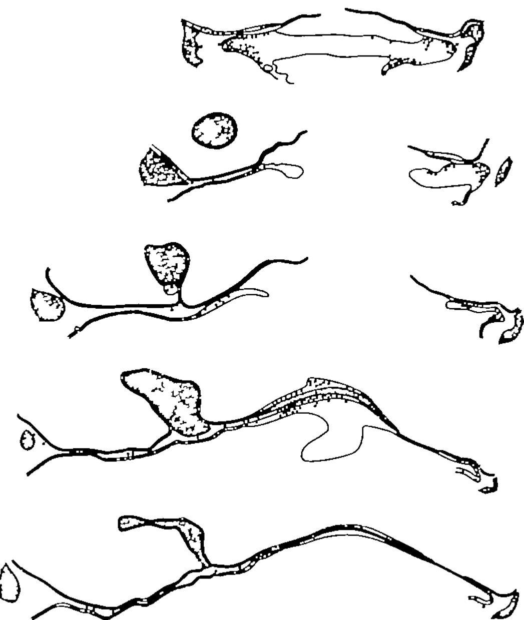
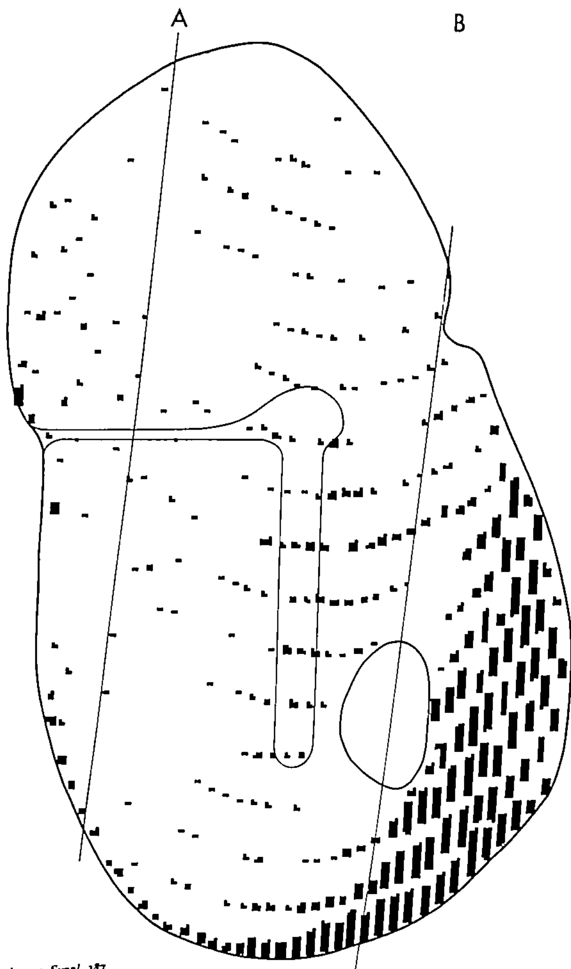


Fig. 83. The course of the thickness of the epithelial layer from the handle of the malleus (H) towards the anulus (A) at various distances from the edge of the perforation after 4 days. (same membrane as in Fig. 89). The ordinate indicates the thickness in μ, the upper traces that on the upper side and the lower that underneath the perforation.


A
B
C
D
E

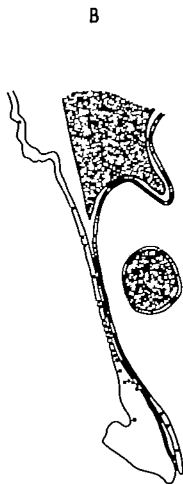


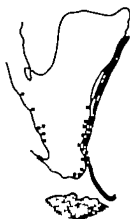
g Rp. Labelled cells after 4 day


A
B

A

B





R10. Labelled cells after 6 days.

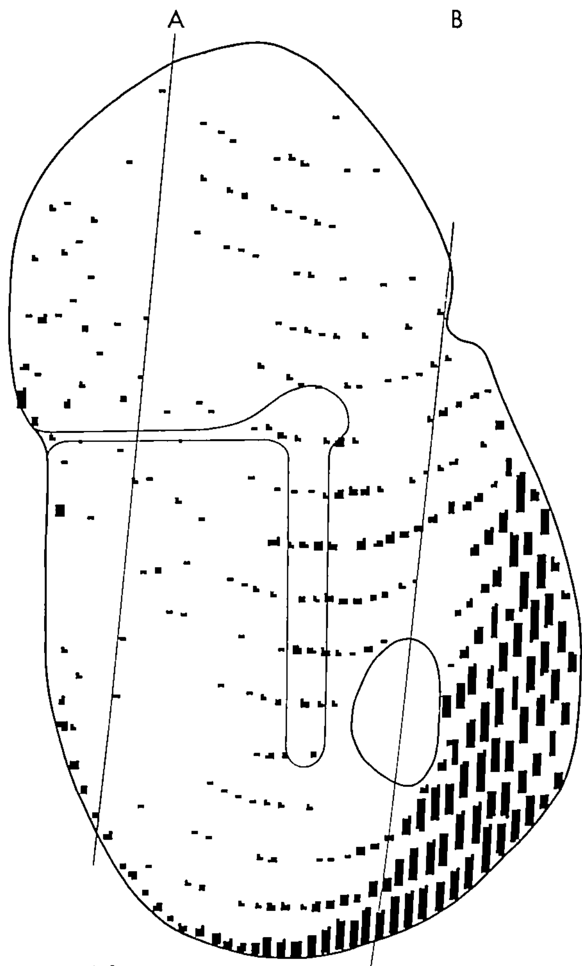
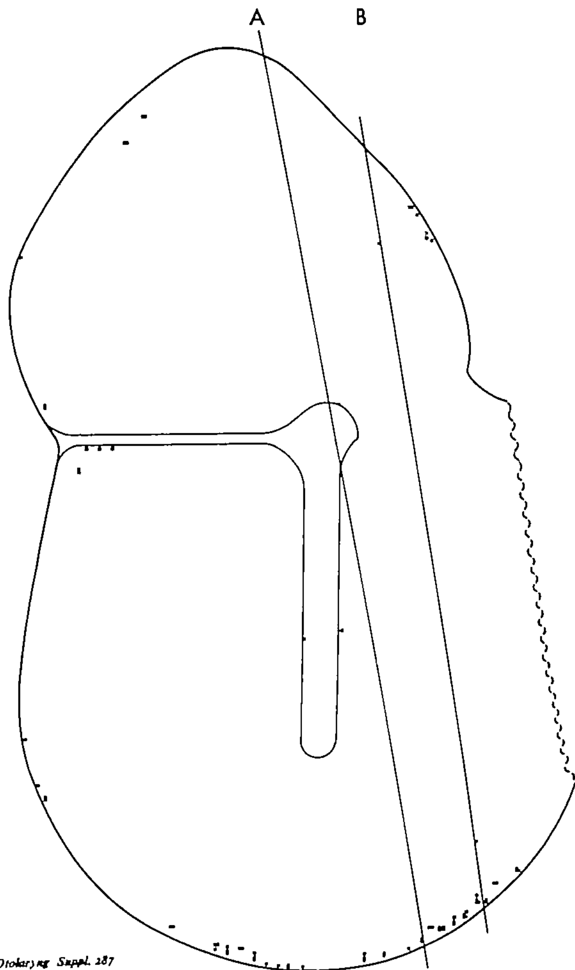

A
B



Fig. 8. Labelled cells after 6 days


A
B

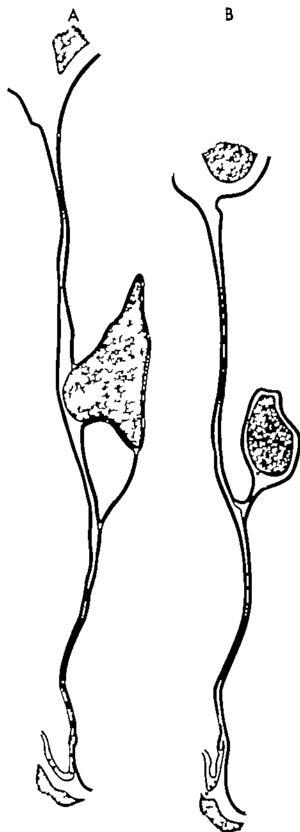
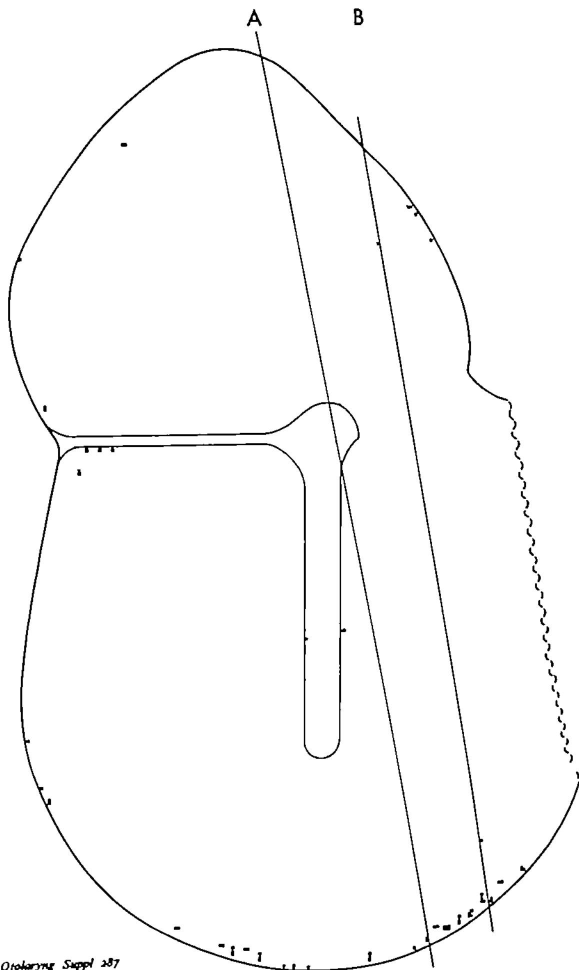


Fig R11 Labelled cells after two weeks. The sections A and B course through the region here the perforation was made.


A
B

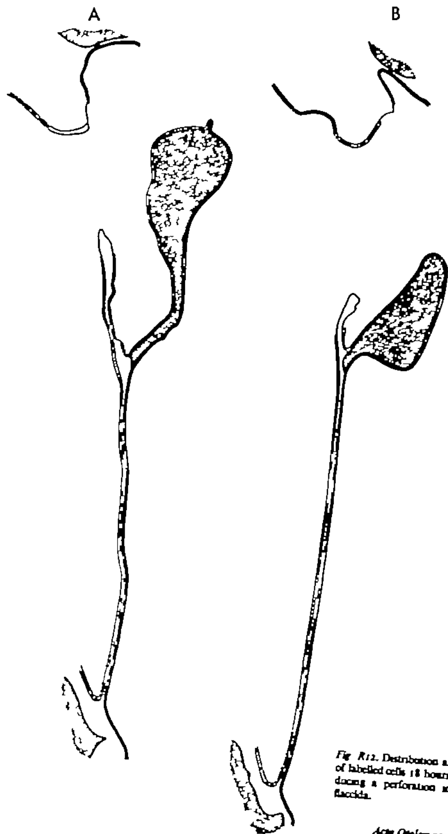
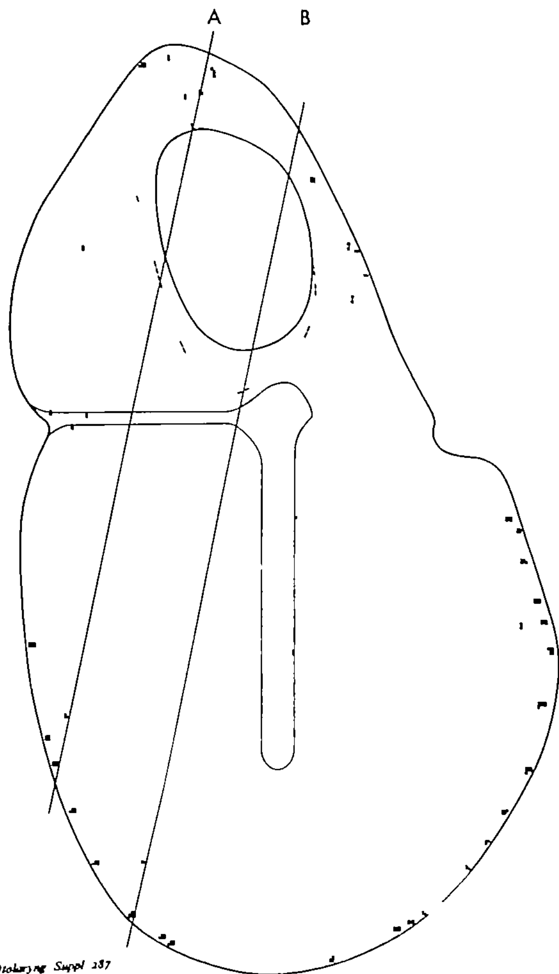


Fig. R12. Distribution and number of labelled cells 18 hours after producing a perforation in the pars flaccida.


A
B

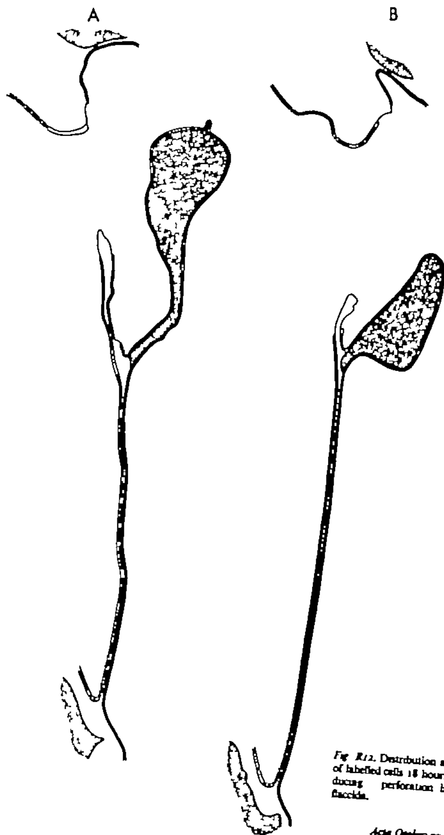
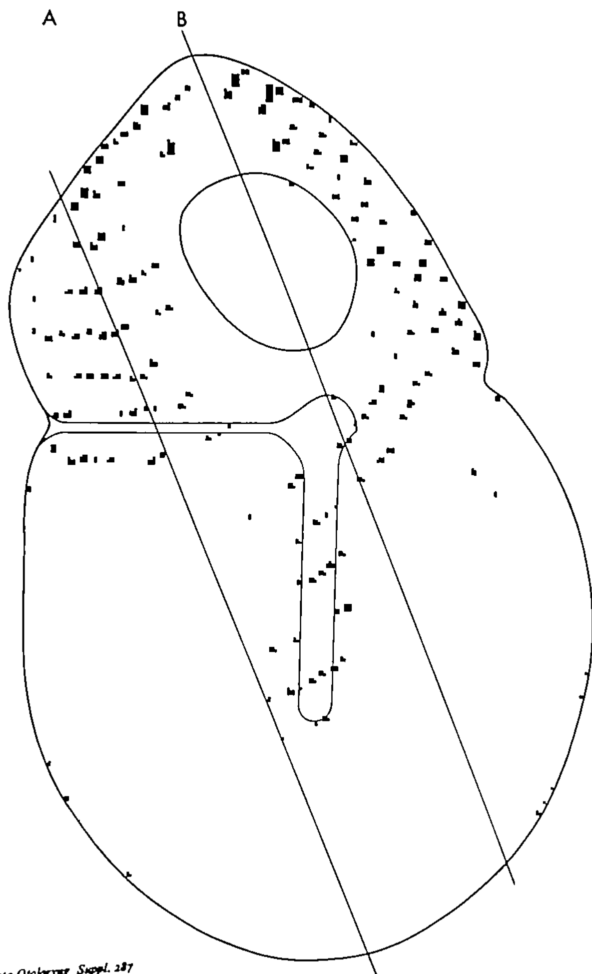


Fig. R12. Distribution and number of labelled cells 18 hours after producing perforation in the pars flaccida.


A
B

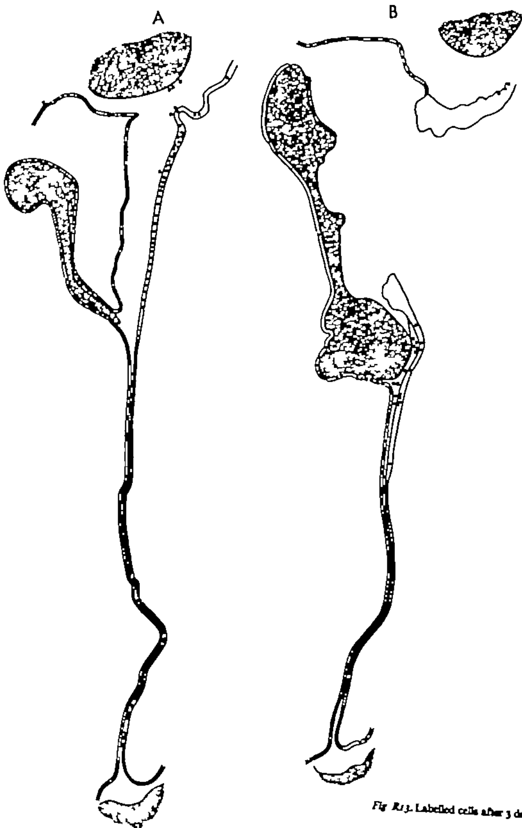


Fig. R13. Labelled cells after 3 days.


A
B
C

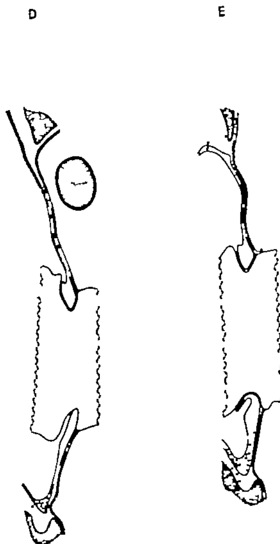


Fig. R14. Sections of drum membrane in which perforation was closed with fat graft, after two days (same membrane as in Fig. 46).

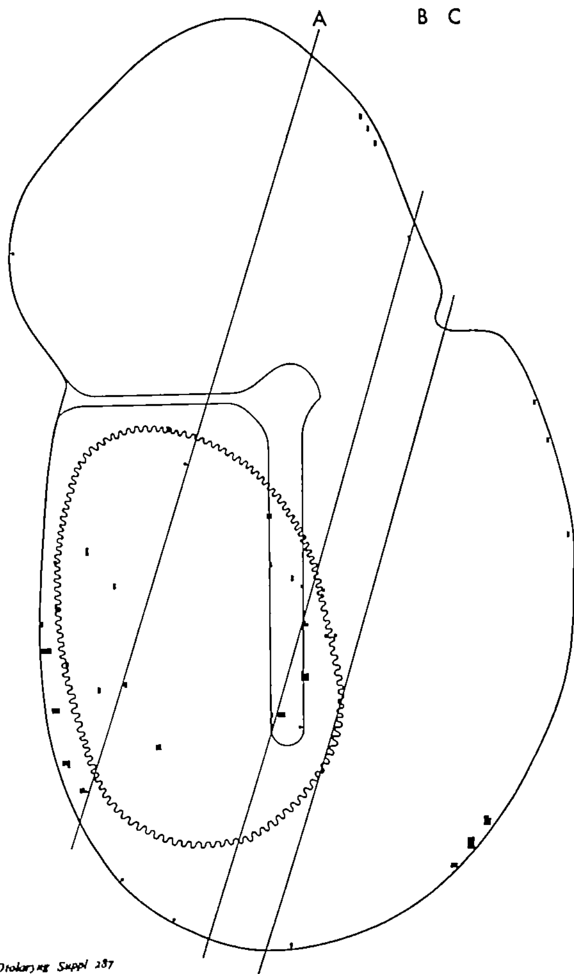

A
B
C



Fig R15. Labelled cells 10 days after a fat graft. The waved line indicates the location of the fat graft incorporated into the membrane. The dash- point line in the sections indicates the demarcation of the subepithelial area, only consisting of vascularised connective tissue, and the fatty tissue.

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SUPPLEMENT 288

Studies of the Distribution of Cochlear Potentials Along the Basilar Membrane

BY

L. U. E. KOHLLÖFFEL

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1 Introduction

Most of our knowledge about the hydromechanical action of the inner ear we owe to the admirable work of Georg von Békésy (1960 a). His direct observations of the basilar membrane vibration established the travelling wave pattern of disturbance as the mode of response by which the cochlea reacts to sound. Upon tonal stimulation of the inner ear a travelling wave appears on the cochlear partition whose wavelength becomes shorter as we proceed from base to apex. The envelope of the displacement wave has a flat basal and a steeper apical slope this gives the envelope its typical asymmetrical shape. The position of the rather flat envelope maximum depends on stimulus frequency it shifts with decreasing frequency progressively from the basal towards the apical end of the cochlea. Observing the excursion of points along the membrane as a function of frequency on Békésy obtained their frequency response curves. When expressed in db per octave the typical slope values of his response curves are 6 db per octave for the low frequency and 20 db per octave for the high frequency branch.

Von Békésy used in his studies stimulus frequencies of not more than 4 kHz, i.e. he worked in the apical, low frequency region of the basilar membrane. Recently the Mössbauer effect was put to use in studies of cochlear mechanics at the basal, high frequency end of the basilar membrane. Johnstone & Boyle (1967) and Johnstone (1969) working on the guinea pig obtained response curves with low frequency slope values of 12 db per octave and high frequency slope values of 70 to 100 db per octave at an SPL around 90 db. Rhode & Geisler (1969) measured in the squirrel monkey slope values of 5 to 10 db per octave on the low frequency side and 90 to 150 db per octave on the high frequency side.

It would seem that these more recent mechanical data conform better to the threshold-frequency response curves of primary auditory nerve fibres obtained by Tasaki (1954 1960) than do the curves of von Békésy However evidence compiled over the past few years on the discharge patterns of primary fibres makes it appear doubtful whether a simple relationship exists between the neural output from segments along the basilar membrane and the corresponding local mechanical input. Ingeniously devised experiments on the neural response to aperiodic stimuli (Kiang, 1965 de Boer 1969 Møller 1970) revealed a transducer mechanism with much less damping than is implied by the strongly damped response of the basilar membrane (Weiss, 1964 Flanagan, 1960 Siebert, 1962). Additionally threshold-frequency response curves (tuning curves) obtained by several investigators (Katsuki et al., 1958 Kiang, 1965 Evans, 1970 a) are relatively narrow over a considerable part of the dynamic range and fibres respond to a wide band of frequencies only at relatively high sound pressures. In view of this discrepancy between the neural output data and the mechanical input data of the cochlear transducer as far as frequency selectivity is concerned it seems necessary to postulate a sharpening mechanism intervening between the mechanical displacement of the membrane and the resulting neural discharges in auditory nerve fibres. However at present mechanical data are only available for stimulus intensities of 90 db SPL or above, i.e. from that part of the dynamic range where neural tuning curves are wide. Thus there is the important question whether

it is permissible to simply extrapolate mechanical data to sound pressures lower than 90 db SPL where the neural response is extremely sharply tuned

Since the study by Tasaki, Davis & Legouix (1952) it is well known that the excitation pattern along the basilar membrane may be investigated in an alternative way to that followed by von Békésy These authors inserted four pairs of differential electrodes into the four turns of the guinea pig s cochlea and they measured the longitudinal distribution of the cochlear microphonic (CM) in response to sinusoidal stimuli The resulting CM distributions and their dependence on stimulus frequency were qualitatively similar to von Békésy s observations on the vibratory pattern of the basilar membrane Legouix (1965/6 1969), using the same technique studied the CM in response to impulsive stimuli His findings are in good qualitative agreement with mechanical data the transient CM response shows the typical strongly damped oscillations with a natural frequency characteristic to the region of the basilar membrane from which an electrode is recording There is good evidence that the CM response is associated with the shearing motion at the hair bearing end of the sensory hair cells (von Békésy 1960 a) and that it originates in the region of the receptor pole of the hair cells (Tasaki et al 1954) That is why we shall use throughout this study the term hair-cell generator when strictly speaking, we only mean the source of the CM in the organ of Corti

Thus there are basically two ways in which the excitation pattern along the basilar membrane may be studied In the one mechanical excursions are measured directly In the other measurement is made of the electrical potentials which are the consequence of the mechanical excursions The greater sensitivity inherent in the electrical method offers the advantage that the CM can be used to explore the excitation pattern in the region of lower sound pressures, i e in the region below 90 db where the minute membrane deflections have not yet been resolved by mechanical methods. The disadvantage of the electrical method, however is one of data interpretation In the past there has been considerable uncertainty on the question how to evaluate exactly CM recordings taken with electrodes from the cochlear fluids. This question concerns the relationship between the potentials generated within the organ of Corti and those picked up by recording electrodes. A constructive step towards a better understanding was taken by Whitfield & Ross (1965) in a theoretical study They defined mathematically the relationship between hair-cell generator potentials and electrode outputs. According to this definition an electrode records the 'weighted sum of the electrical activity along the membrane. This, it is argued is due to the fact that hair-cell potentials spread out and interfere with each other to a considerable extent in the inner ear fluids. Thus the spatial spread of signals in the cochlea was recognised to be instrumental to the quantitative interpretation of CM data and a reappraisal of CM measurements seemed appropriate

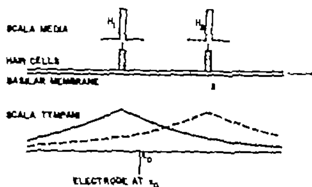


Fig 1.1 Diagram to show the interference of hair-cell outputs (H_1 and H_2) in the cochlear fluids due to signal spread along the canals. Electrode at x_0 records a potential which is the sum of the attenuated contributions from hair-cell generators at x_1 and x_2.

The present investigation developed logically from the mathematical formula relating hair cell output to electrode output In Fig. 1 1 two hair-cell generators are schematically shown, situated on the basilar membrane at points x_1 and x_2 respectively Let H_1 be the electrical

output at x_1 and H_2 that at x_2. H and H_2 are represented as spatial impulses. Each hair-cell generator establishes a potential field according to the conductive properties of inner ear tissues and fluids. The decay of potential strength with longitudinal distance from the generating source is indicated for scala tympani by an exponential attenuation function. It can be clearly seen from the illustration that an electrode at point x_e records a potential which is the sum, or superposition of the contributions from the generators at x and x_2. In the case of n hair-cell generators we obtain as expression for the electrical output at x_e

$$CM(x_e) = \sum_i H_i(x_i) w(x - x_i)$$

$w(x_e - x_i)$ is a weighting factor which takes account of the signal attenuation between the point of generation x_i and the recording site x_e. We may conceive of the basilar membrane as infinitely densely packed with hair-cell operators and consequently arrive at an integral expression for the CM at x_e.

$$CM(x_e) = \int_{-\infty}^{\infty} H(x)\, W(x_e - x)\, dx \qquad (1)$$

Equation (1) is fundamental to the present study. It defines the relationship between the hair-cell output pattern along the basilar membrane, $H(x)$, and the resulting CM distribution along the cochlear scalae, $CM(x_e)$. $W(x_e - x)$ is a weighting function which brings into play the signal attenuation effective between the points x and x_e. The expression in equation (1) is a convolution integral and by analogy to linear communication theory we may call the connection between $H(x)$ and $CM(x_e)$ a 'spatial filter operation'. Hence $H(x)$ is the input function and $CM(x_e)$ the corresponding output function. $W(x)$ is the spatial impulse response of the system.

Experiments to be described here were designed to examine the validity of equation (1). A suitable starting point seemed to be the study of CM distributions in response to high frequency tones. Such stimuli cause disturbances only in the basal coil of the cochlea and it seemed possible to measure very accurately and completely the corresponding CM patterns with a multi-electrode array arranged along the basal cochlea. At high frequencies the travelling waves have very short wavelengths and thus closely neighbouring hair cells are driven out of phase. One might expect therefore that phase cancellation is much more pronounced and that the spatial filter effect can be seen much better at high frequencies than at low ones.

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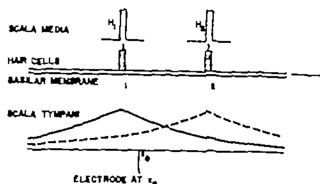


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The present investigation developed logically from the mathematical formula relating hair cell output to electrode output. In Fig. 1.1 two hair-cell generators are schematically shown situated on the basilar membrane at points x_1 and x_2 respectively. Let H_1 be the electrical

our case, outweigh the advantage of recording potentials larger by 6 db

The problem of arranging a set of recording electrodes over a short length of the basilar membrane seemed to be most elegantly tackled by the use of a multi-electrode probe manufactured with the modern techniques of micro-miniaturization. Such a probe was manufactured for this project in the Lucas Research Department, Shirley Birmingham. Twelve narrow stripes of aluminium were photoetched onto a glass substrate which was cut into a rectangular shape appropriate for the insertion of the probe through a slit into scala tympani. However this approach proved unsuccessful because the probe was brittle and much too delicate to be repeatedly used in a large series of experiments. Thus very early on, during the exploratory phase of the project, the probe broke and this technique was discontinued. After this failure an alternative technique was adopted. A series of up to twelve holes was drilled into the wall of the basal scala tympani parallel to the basilar membrane and stainless steel wire electrodes were inserted into the holes. The placement of electrodes was extremely time-consuming. This method was finally abandoned in favour of the ultimate, optimum technique which allowed the rapid collection of data. A multi-electrode array was constructed by gluing insulated stainless steel wires together The elastic, ribbon-like array thus formed could be easily inserted through a slit into scala tympani alongside the basilar membrane

SURGICAL PROCEDURE

All guinea pigs were anaesthetised with Urethane administered intraperitoneally at a dose of 1.25 g kg. Tracheotomy was performed and a short polythene cannula was inserted into the trachea. The bulla on the animal's right side was exposed in the standard latero-ventral fashion described by Tasaki & Fernández (1952). Then the animal was mounted in a stereotaxic instrument with the head rigidly clamped between the skull and the upper jaw bone in a head holder The external auditory meatus was exposed and cut across leaving only a short sleeve for the insertion of the coupling tube of the earphone.

With a number of wire hooks the tissue surrounding the bulla was carefully retracted. The bulla was opened with a dental drill and part of the bony shell was removed with a forceps. Thereafter the cochlea protruding into the middle ear cavity was conveniently accessible for surgical manipulation. With small pieces of cotton wool the surface of the cochlea was cleaned and dried.

After these preliminary operations microdissection began with the aid of a Zeiss otoscope which both illuminated and magnified the operative field. With a small, round dental burr (1/4 C. Ash & Sons) the wall of the basal 1/2 turn of scala tympani was thinned down to uniform thickness along the course of the basilar membrane. The thickness was reduced until the bony wall was almost transparent. This procedure had to be executed with utmost care. Accidental sideways slipping of the burr by only about 1 mm and subsequent injury to the retracted tissue in the immediate vicinity could cause bleeding and filling of the middle ear cavity with blood. Removal of blood clots especially from the stapedial region was very delicate. It was found empirically that guinea pigs weighing about 500 g possessed cochlear walls with the optimum mechanical consistency for this operation. Guinea pigs below this weight had cochleae too fragile and heavier animals usually had a bony shell too hard and brittle. In either extreme case it could easily happen that the cochlear shell broke during the drilling process.

In the early experiments (single electrode technique) a number of holes were drilled with a stiff needle into the wall of scala tympani parallel to the basilar membrane. Since it was desirable to accomplish a fairly regular spacing of the holes the needle was mounted in a heavy handle. The weight of the handle stabilized the needle once it was placed on the

2 Methods

For the study of the spatial filter effect it was necessary to obtain a detailed picture of the CM distribution over a sufficient length of the cochlea. Analysis of the microstructure of CM patterns with equation (1) seemed only a *promising enterprise provided accurate* and reliable data about the longitudinal course of the CM were made available. The accommodation of an assembly of closely spaced electrodes allowing the detailed inspection of CM patterns was the central problem in the development of suitable techniques.

The set of recording electrodes was placed *in the basal scala tympani of the guinea pig's* cochlea. This site was chosen for mainly two reasons. First this part of the cochlea appeared to be spacious enough to allow the insertion of several electrodes without damage to the cochlear partition. Second the CM response to high frequency stimuli was expected to show the spatial filter effect most clearly. It was therefore imperative to execute measurements in the basal area where the response to such stimuli is known to occur.

The choice of the basal cochlea as recording site carried the advantage that the conditions of current spread have been studied most extensively for this part of the cochlear ducts (see Section 3 Weighting Function). As to the question which recording technique *should* be used, the differential technique with a set of paired electrodes along the cochlear partition or the simpler technique with a set of electrodes in one scala and a common reference electrode outside the cochlea, e.g. on the animal neck, there seemed to be no objection from existing evidence to using the simpler *technique. For CM recordings from* the upper turns of the guinea pig's cochlea most workers have preferred the differential technique, i.e. they recorded the potential difference between one electrode in scala tympani and one opposite in scala vestibuli. It has been suggested in the literature (Tasaki et al. 1952, Tasaki & Fernández, 1952) that the differential technique is superior to the alternative of measuring potentials between one electrode in scala tympani and the other on the neck. A differential pair of electrodes is supposed to record potentials only from one turn without contamination by signals arising from neighbouring turns. However Tasaki & Fernández felt that for the basal coil such a superiority did not exist and they stated: The microphonic recorded with one electrode on the neck and the other in the basal turn, either in scala vestibuli or in scala tympani, gives information as to the activity taking place in the basal turn without contamination by the responses originating in the upper turns. Recently Dallos (1969 *a*) confirmed this statement in a thorough study. Apart from the fact that the differential technique recorded potentials larger by 6 db in the basal turn he could not find any advantage over the simpler technique, i.e. one electrode in the basal scala tympani and the reference on the neck. In our case the simpler technique is preferable for mainly two reasons. 1) The *technical difficulties in setting up a differential* recording situation with twelve closely spaced pairs of electrodes are most probably prohibitive. 2) Cross-conduction between scala vestibuli and scala tympani via the fluid leaking out of the openings in the cochlear wall would probably be unavoidable. This would introduce uncontrollable current loops short-circuiting the cochlear partition. Thus it is clear that the disadvantages of the differential technique in

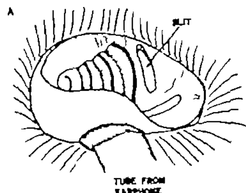
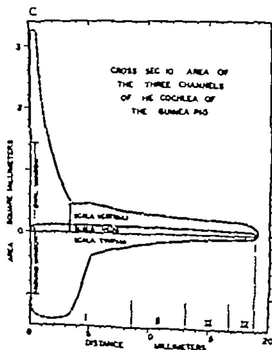




Fig. 2.1 (A) Schematic drawing of the exposed right cochlea of the guinea pig. The slit in the wall of the basal 1/2 turn of scala tympani is shown. (It is in the experiments approximately 2 mm long and 0.5 mm wide.) The slit runs parallel to the basilar membrane. The position of the stria vascularis is marked as a dark band.

(B) Right cochlea of the guinea pig with organ of Corti exposed. This photograph was taken from Engström et al. (1964). The schematic representation of the electrode array tips was superimposed on the photograph in order to show the approximate array position relative to the basilar membrane.

(C) Cross section area of canals according to Fernández (1952). The slit in the wall of scala tympani would—when plotted in this graph—extend from about 3 to 5 mm along the abscissa. The most basal electrode of the array 1 would be located between 3 to 3.5 mm.



completely and there was no danger of further formation of blood clots. Finally the Ringer fluid was sucked out of the middle ear cavity without, however removing fluid from scala tympani.

Then with a micromanipulator arrangement the multi-electrode array was inserted through the slit into scala tympani to a depth of about 300 to 400 μ. The tips of the recording electrodes were thus located in a line running at a distance of about 500 μ parallel to the basilar membrane.

THE MULTI-ELECTRODE PROBE

A. Manufacture of the array

The multi-electrode probe was the central piece of equipment for the measurement of

bony wall and prevented sideways slipping Up to twelve separate holes could be drilled in this way each with a diameter of about 70–100 μ Insulated 50 μ stainless steel wire electrodes were inserted into the holes. The insulation coating formed small blobs along the shaft of the electrodes and 400 μ away from a suitably shaped blob the electrodes were cut before insertion. Thus each individual electrode was selected and matched to a particular hole, the blob serving as depth control and as closure of the hole The steel wires had just the right combination of flexibility and stiffness which was essential for the successful placement of a set of electrodes. Thus previous attempts with more flexible silver electrodes failed The steel electrodes were about 50 mm long and were soldered to slim cylindrically shaped handles made of brass. After an electrode was inserted into scala tympani the brass handle was pushed into a complementary brass cylinder arranged in a perspex block (see Fig 2 6). The elastic force due to the bending of the electrode between the points of fixation—i.e. the hole in scala tympani and the perspex block—pressed the electrode slightly against the hole and thus held the electrode in a stable position This stabilization was extremely important since during the course of inserting electrodes it was impossible not to touch and disturb electrodes already in place it is only by virtue of the springiness of the steel wires that electrodes stayed in place despite disturbances The minimum distance between the holes could not be reduced below 0 3 mm without breaking the bony ridges between holes. With the need for more closely spaced electrodes the single electrode technique was abandoned in favour of the multi-electrode technique

In the latter technique a multi-electrode array was inserted into a slit like opening in the wall of scala tympani. The slit was manufactured in one of two ways. Drilling of a series of adjacent holes by means of a steel needle and subsequent breaking of the bony bridges between holes with a fine forceps was the one way As alternative to this indirect method there was the direct way of drilling the slit with a small, round dental burr (1/4 C. Ash & Sons). Extreme care and an absolutely steady hand were prerequisite for the success of this procedure. It was necessary to widen the slit so formed in both cases with a fine forceps. The shape of the slit was made to match the dimensions of the electrode array by cautiously breaking minute pieces of bone from the rims of the slit Care had to be taken not to approach too closely the region of the attachment of the basilar membrane to the wall of scala tympani The slit had a length of about 2 mm and a width of about 0.5 mm. It extended from about 3 to 5 mm along the basilar membrane measuring from the basal end of the membrane (see Fig. 2.1 A, B C).

Small blood vessels lining the floor of scala tympani opposite to the basilar membrane were usually injured during the formation of the slit. As a consequence blood clots formed inside scala tympani and it was clear that these clots would seriously distort the pattern of electrical conductivity in the scala. Attempts to remove the blood clots with a fine forceps were usually fatal because the sharp prongs of the forceps easily pierced the thin bony floor of scala tympani towards the modiolar core. In these cases the CM recorded from the basal scala tympani had a very low amplitude and there was practically no longitudinal phase variation Obviously the accidental hole in the modiolar wall effectively earthed scala tympani via the VIII nerve (see von Békésy 1960 a p 660). In order to remove the blood clots from the duct another procedure was adopted. The middle ear cavity was filled with mammalian Ringer solution so that the slit in scala tympani was well below the fluid level. A syringe also filled with Ringer solution, was held over the slit and the plunger of the syringe was slowly moved inwards and outwards. This produced streaming of fluid and effectively washed out the blood clots from scala tympani This procedure was repeated several times until it was certain that bleeding had ceased

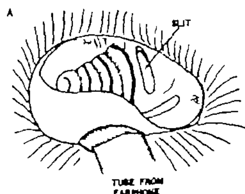




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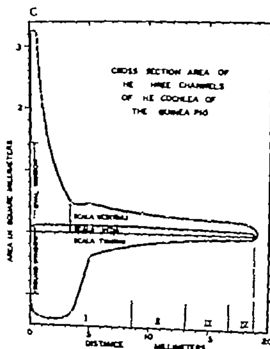
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bony wall and prevented sideways slipping. Up to twelve separate holes could be drilled in this way each with a diameter of about 70–100 μ Insulated 50 μ stainless steel wire electrodes were inserted into the holes. The insulation coating formed small blobs along the shaft of the electrodes and 400 μ away from a suitably shaped blob the electrodes were cut before insertion Thus each individual electrode was selected and matched to a particular hole, the blob serving as depth control and as closure of the hole. The steel wires had just the right combination of flexibility and stiffness which was essential for the successful placement of a set of electrodes. Thus previous attempts with more flexible silver electrodes failed. The steel electrodes were about 50 mm long and were soldered to slim cylindrically shaped handles made of brass After an electrode was inserted into scala tympani the brass handle was pushed into a complementary brass cylinder arranged in a perspex block (see Fig 2 6) The elastic force due to the bending of the electrode between the points of fixation—i e. the hole in scala tympani and the perspex block—pressed the electrode slightly against the hole and thus held the electrode in a stable position. This stabilization was extremely important since during the course of inserting electrodes it was impossible not to touch and disturb electrodes already in place it is only by virtue of the springiness of the steel wires that electrodes stayed in place despite disturbances. The minimum distance between the holes could not be reduced below 0.3 mm without breaking the bony ridges between holes. With the need for more closely spaced electrodes the single electrode technique was abandoned in favour of the multi-electrode technique

In the latter technique a multi-electrode array was inserted into a slit-like opening in the wall of scala tympani The slit was manufactured in one of two ways. Drilling of a series of adjacent holes by means of a steel needle and subsequent breaking of the bony bridges between holes with a fine forceps was the one way As alternative to this indirect method there was the direct way of drilling the slit with a small round dental burr (1/4 C. Ash & Sons) Extreme care and an absolutely steady hand were prerequisite for the success of this procedure It was necessary to widen the slit so formed in both cases with a fine forceps. The shape of the slit was made to match the dimensions of the electrode array by cautiously breaking minute pieces of bone from the rims of the slit. Care had to be taken not to approach too closely the region of the attachment of the basilar membrane to the wall of scala tympani. The slit had a length of about 2 mm and a width of about 0.5 mm. It extended from about 3 to 5 mm along the basilar membrane measuring from the basal end of the membrane (see Fig 2.1 A, B, C).

Small blood vessels lining the floor of scala tympani opposite to the basilar membrane were usually injured during the formation of the slit. As a consequence blood clots formed inside scala tympani and it was clear that these clots would seriously distort the pattern of electrical conductivity in the scala. Attempts to remove the blood clots with a fine forceps were usually fatal because the sharp prongs of the forceps easily pierced the thin bony floor of scala tympani towards the modiolar core. In these cases the CM recorded from the basal scala tympani had a very low amplitude and there was practically no longitudinal phase variation Obviously the accidental hole in the modiolar wall effectively earthed scala tympani via the VIII nerve (see von Békésy 1960 *a* p 660) In order to remove the blood clots from the duct another procedure was adopted The middle ear cavity was filled with mammalian Ringer solution so that the slit in scala tympani was well below the fluid level. A syringe also filled with Ringer solution, was held over the slit and the plunger of the syringe was slowly moved inwards and outwards. This produced streaming of fluid and effectively washed out the blood clots from scala tympani This procedure was repeated several times until it was certain that bleeding had ceased

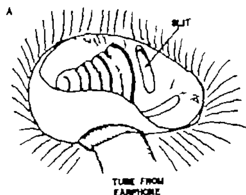
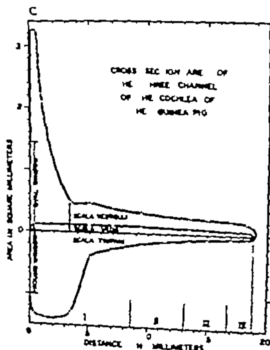




Fig. 2 1 (A) Schematic drawing of the exposed right cochlea of the guinea pig. The slit in the wall f the basal 1/2 turn of scala tympani is shown. (It is in the experiments approximately 2 mm long and 0.5 mm wide.) The slit runs parallel to the basilar membrane. The position of the stria vascularis is marked as dark band.

(B) Right cochlea of the guinea pig with organ of Corti exposed. This photograph was taken from Engström et al. (1966). The schematic representation of the electrode array tips was superimposed on the photograph in order to show the approximate array position relative to the basilar membrane.

(C) Cross section area of canals according to Fernández (1952). The slit in the wall of scala tympani *would—when plotted in this graph—extend from* about 3 to 5 mm along the abscissa. The most basal electrode of the array 1 would be located between 3 to 3.5 mm.



completely and there was no danger of further formation of blood clots. Finally the Ringer fluid was sucked out of the middle ear cavity without, however removing fluid from scala tympani.

Then with a micromanipulator arrangement the multi-electrode array was inserted through the slit into scala tympani to a depth of about 300 to 400 μ. The tips of the recording electrodes were thus located in a line running at a distance of about 500 μ parallel to the basilar membrane.

THE MULTI ELECTRODE PROBE

A. Manufacture of the array

The multi-electrode probe was the central piece of equipment for the measurement of

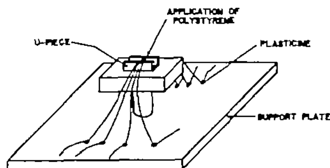


Fig 2.2 Schematic drawing of the wire assembly arrangement.

the longitudinal distribution of the CM response The individual electrodes composing the probe were made of the same 50 μ stainless steel wire which was used in the single electrode technique. Steel wires, about 150 mm long were slowly drawn through a bath of Bakelite Insulating Lacquer The coat so formed was then baked for not less than 20 min in an oven at 180 C. Wires were given at least three coats.

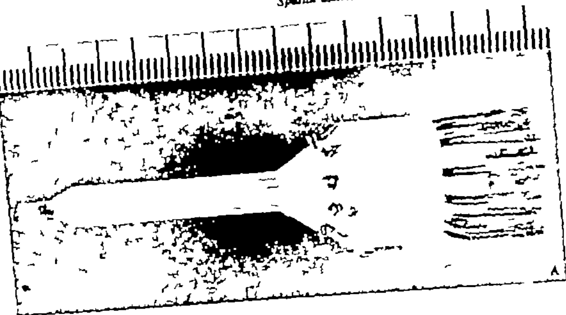
Twelve insulated wires were assembled side by side in a single plane and they were bonded together with polystyrene. The assemblage was carried out under visual control (Zeiss otoscope) In order to achieve an accurate alignment of the electrodes in a single plane the wires were pulled over the edges of a U shaped piece of metal. A schematic drawing of the simple assembly arrangement is given in Fig 2.2. The procedure began by tightly pulling the first insulated wire over the U piece and fixing the wire ends on the 'support plate with plasticine The second wire was arranged in the same way close to the first one With a paint brush a small droplet of a polystyrene benzene solution was spread along the electrode wires over a length of about 15 mm Due to the surface tension of the liquid the wires were pulled close together The polystyrene dried rapidly and fixed the wires. Then the third electrode was bonded to the second one and the manipulation was repeated until all twelve electrode wires were glued together In the event that any particular wire within the set was not aligned properly a small drop of benzene was applied so that the polystyrene softened locally This made a realignment of the wire possible.

The distance between adjacent electrode wires was mainly determined by the blob-like thickenings of the insulation coating along the wires. It was possible to achieve a regular spacing of electrode wires in the array by carefully selecting wires with approximately equal blob sizes. Thus the distance between the centres of adjacent electrodes varied from about 150 to 180 μ Since the electrode diameter was 50 μ this variation in distance was considered tolerable.

B. Mounting of the electrode array

One end of the assembly of wires was trimmed with scissors and the completed ribbon like array was then mounted on the electrode probe carrier' (see Fig. 2.3 A) A rectangular piece of ordinary film tape was glued to the small metal lip sticking out of the carrier shaft. The electrode array was bonded with Eastman adhesive 910 to this flexible tape support in such a way that about 8 mm of the ribbon like array protruded beyond the support. A schematic drawing of this arrangement is given in Fig. 2.3 B The free ends of the electrode wires were embedded with polystyrene in twelve grooves cut lengthwise into the round shaft of the carrier Finally after scraping off the insulation from the terminal section with a scalpel the electrode wires were soldered to twelve small steel bars permanently attached to the upper end of the carrier The carrier was made of heat resisting Teflon to avoid melting of carrier material during soldering

The carrier was then connected to the main part of the array holding unit (see Fig 2.4). This part consisted of a round perspex shaft with twelve permanently embedded steel wires leading to twelve preamplifiers arranged at the top end of the shaft. A short, cylindrical piece of perspex was inserted between the probe



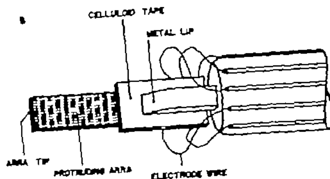


Fig. 2.3 (A) Photograph of the 'electrode probe carrier' (scale division in mm).

(B) Schematic drawing of the tip of the 'electrode probe carrier' with the electrode array mounted.

carrier and the main shaft. It contained the electrical components necessary for the a.c. coupling between electrodes and preamplifiers. (For the measurement of cochlear d.c.-potentials this a.c.-coupler could be removed. In the experiments reported here no d.c.-measurements were undertaken and the a.c.-coupler was always in place.)

The shape of the complete electrode array holding unit represented a compromise between two partly conflicting design criteria. It was desirable to make the twelve wires leading away from the recording site as short as possible in order to minimize capacitive cross-talk i.e. the preamplifiers had to be as close as possible to the recording site. On the other hand the preamplifiers had to be accommodated at a sufficient distance from the operative field not to obstruct the experimenter's vision.

C. Insertion of the electrode array into scala tympani

The alignment of wires was checked under the Zeiss otoscope before insertion of the tip of the electrode array into scala tympani. During the course of three to four experiments usually small pieces of polystyrene broke away from the tip of the array causing an irregular spacing of electrode terminals. By carefully cutting the tip of the array with a pair of scissors it was possible to use the remaining intact part of the array for further experiments. In this way the same array could be used for about ten successive experiments.

For the placement of the array in scala

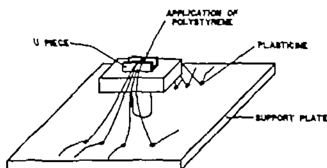


Fig 2.2 Schematic drawing of the wire assembly arrangement.

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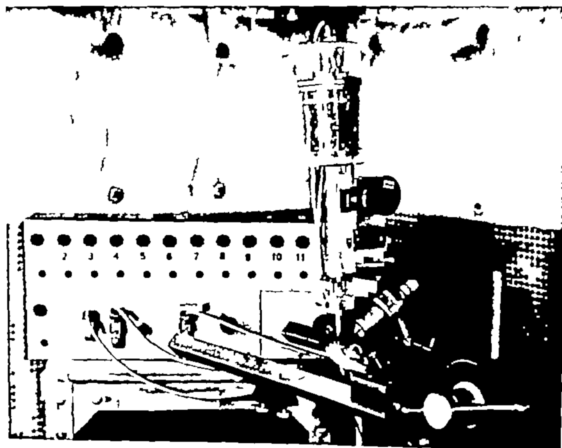


Fig. 2.5. Photograph showing the electrode array holding unit in the experimental position on the stereotaxic instrument. The array tip is inserted into the basal scala tympani of the right cochlea in the guinea pig skull. The holding unit is coupled via the ball joint to manipulator II. Preamplifiers on the holding unit are connected to the twelve main amplifiers contained in the box, shown in the background. The screened condenser earphone with the short metal tube for the acoustic coupling is displayed next to the guinea pig skull.

ELECTRICAL PROPERTIES OF THE ELECTRODE CHANNELS

It was essential for the faithful measurement of the CM distribution in the basal scala tympani that all data channels had identical electrical characteristics. In Fig. 2.7 one such channel is shown schematically representative for the whole set of the twelve parallel channels. The electrode wire was a.c.-coupled to the preamplifier. (Both a.c.-coupler and preamplifier were attached to the array holder.) The preamplifier was connected with a cable to the main amplifier stage contained in a separate box. The preamplifier in series with the main amplifier formed the 'channel amplifier'.

Recordings were taken between the sensing electrodes in scala tympani and an indifferent electrode on the animals neck. Hence the 'internal impedance' of the signal source was the sum of two components. The one component was the impedance between the basal scala tympani and animal earth which according to von Békésy (1960 *a*, p. 659) had a value of about 8 kΩ. The other component was the impedance at the electrode-perilymph interface. (The insulation coating was absent only at the cross section of the tip of the steel electrode.) This impedance amounted to ap-

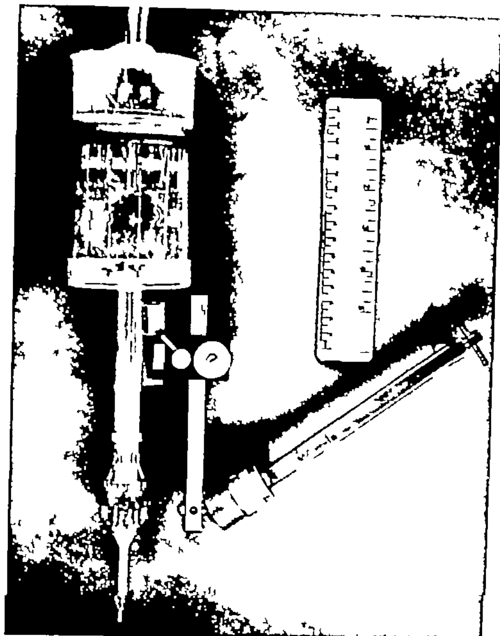


Fig 2 4 Photograph of the electrode array holding unit and preamplifiers. The small, ribbon-like electrode array is shown at the tip of the unit. The holding unit is mounted on manipulator I which in turn is coupled via the ball joint link (shown in the photograph) to manipulator II (shown in Fig. 2.5)

tympani a combination of two micromanipulators was employed (see Fig 2.4 and Fig 2.5) Manipulator I was coupled to manipulator II via a ball and socket joint this gave sufficient freedom of movement to manoeuvre the array tip into scala tympani through the narrow slit in the cochlear wall. The main shaft of the array holding unit was screwed to manipulator I With the ball joint unlocked manipulator I was tilted in such a way that the electrode array tip was in line with the slit in the basal turn Then the ball joint was locked and the electrode array position was adjusted in the lateral directions with manipulator II Finally the array was driven into scala tympani with manipulators I and II

D Channel connections in the single-electrode technique

In the single-electrode technique all electrodes were mounted in a perspex block (see Fig 2.6) With short leads connection was made to the a.c.-coupler attached to the main shaft of the array holding unit. Thus in both techniques the same channel amplifiers were employed for the recording of potentials.

fier channels. Second, since all channel amplifiers were fed from the same power supply there was a possibility of a channel interaction via the internal impedance of the power supply. This problem was solved by choosing a power supply unit with a small internal impedance.

Channel interaction was determined by inserting a test signal into one data channel and measuring the response in the idle adjacent channels. It turned out that the channel interaction could be considered negligible for our purposes. The response in the idle channels was maximally only about 1 to 1.5% of the response in the test channel and this was at the highest frequency employed in the study (20 kHz).

SOUND GENERATING EQUIPMENT

The stimulus signal was derived from two Solartron oscillators (Type CO 546) each of which was equipped with an attenuator covering a range of 60 db. The signal was fed via a 600Ω adaptor to the microphone amplifier. The latter was built in this laboratory. It had a flat frequency response from 100 Hz to 30 kHz and a gain factor of 29. Its linear dynamic range extended up to an output voltage of about 55 V rms. The output of the microphone amplifier was connected to the one-inch condenser microphone (Bruel & Kjaer 4132) which was used in the experiments as earphone, i.e. as electro-acoustic transducer. The voltage (190 V d.c.) for the polarization of the condenser microphone was supplied by the microphone amplifier.

The acoustic output of the one-inch condenser microphone was coupled via a short piece of metal tubing (length 15 mm) to the guinea pig's external ear. The transducer was carefully screened in order to prevent direct pick-up of the stimulus signal by the recording electrodes.

The calibration of the sound equipment was executed by monitoring the sound pressure in front of the eardrum with the aid of a calibrated sensing probe connected to a half-inch condenser microphone (Bruel & Kjaer 4134). The output of the sensing microphone was fed to a Bruel & Kjaer Microphone Amplifier Type 2604. It was found from this measurement that the sound generating system had a reasonably flat frequency response. Thus, there was an SPL at the eardrum of 90 ± 3 db from 200 Hz to 10 kHz for an oscillator output of 4 V rms and an attenuator setting of 25 db. The SPL had a value of 100 ± 5 db in the frequency range from 10 kHz to 70 kHz. (The standard reference level for the sound pressure is 0.0002 dynes per cm^2.)

The condenser microphone introduced quadratic distortion so that the second harmonic of the stimulus frequency appeared in the acoustic signal. However, this effect could be neglected in the present study since the second harmonic was smaller than the actual signal by 40 db at stimulus intensities usually employed (80 to 90 db SPL).

RECORDING

The block diagram of the recording system is shown in Fig. 2.8. The input, output and reference channels are marked differently for reasons of clarity.

The animal was placed in a shielded, soundproofed room. A sinusoidal stimulus from oscillator O_1 was applied to the guinea pig's ear by closing push-button switch S. (In two-tone interaction and overstimulation studies oscillator O_2 was used as well.) The resulting CM responses were amplified by the twelve a.c.-coupled channel amplifiers. By means of switch S_2 a particular output channel was selected. The output was then fed through a B & K amplifier and 1/3 octave filter (Bruel & Kjaer Microphone Amplifier Type 2604, Bruel & Kjaer Band-Pass Filter Type 1612). The unfiltered output could be directly displayed on a dual-beam oscilloscope (Tektronix Type 502 A) for inspection of the waveform of the CM.

For a given stimulus all twelve outputs were measured, one at a time. The amplitude of the

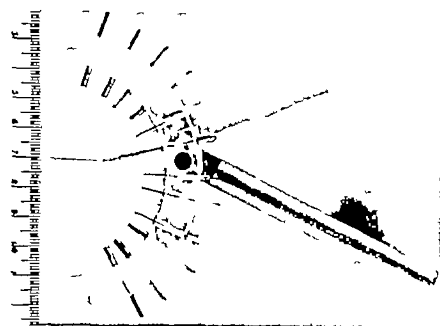


Fig 2.6 Photograph of the ring-shaped perspex block for the *mounting of* electrodes in the single electrode technique. The handle of the electrode wire (shown in the inside of the ring) is pushed into the complementary brass cylinder embedded in the block. The electrode wire thus mounted is then connected with a lead (shown on the outside of the ring) to a channel amplifier (scale division in mm)

proximately 30 kΩ. Due to the high input resistance of the preamplifiers (22 MΩ) there was no danger that slight differences in the internal impedance of the twelve channel sources would distort the measurement of the CM pattern. In addition the high input resistance of the preamplifiers served a further purpose. It was important not to introduce with the sensing equipment current loops of high conductivity which could alter the conductivity pattern present in the cochlea. The high input impedance of the data channels prevented such a disturbance of the current distribution in scala tympani.

All twelve transistorized amplifiers were built identically and they had the same flat frequency response from 0 to 20 kHz. The 3 db cut-off frequency was 40 kHz. Since electrodes were a.c.-coupled to the amplifiers a lower 3 db cut-off frequency of about 10 Hz was introduced. Thus over the frequency range (500 Hz to 20 kHz) used in the study the frequency response of the amplifiers was flat. The gain factor of the channel amplifiers was set at 500. However despite careful adjustment there remained a slight difference of maximally 2% between the gain factors of different channels.

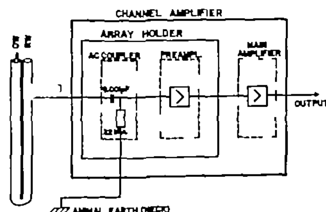


Fig 2.7 Block diagram of one channel amplifier representative for all twelve channel amplifiers. (The schematic drawing on the left shows the twelve electrodes of the array inserted into scala tympani, *RW* = round window *OW* = oval window)

A problem of major concern in the design and construction of electrode channels was the minimization of channel interaction. Only in the case of negligible interaction would channel outputs represent a replica of the original potential distribution in the cochlea. There were two potential sources of significant electrical interaction of the individual channels. First there was the interaction due to capacitive cross-talk. Its influence was kept at a minimum by making the wires from the recording site to the preamplifiers as short as possible and by screening the individual ampli

fier channels. Second, since all channel amplifiers were fed from the same power supply there was a possibility of a channel interaction via the internal impedance of the power supply. This problem was solved by choosing a power supply unit with a small internal impedance.

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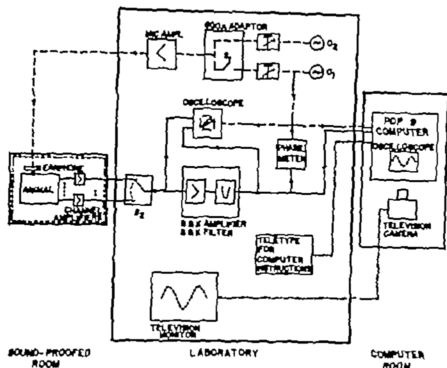


Fig. 2.8 Block diagram of the experimental set-up. - - - stimulus signal —— CM output signal — reference signal (from stimulus); —·— Computer instructions and television link.

filtered CM response was read from the monitor of the B & K amplifier. The phase was determined in one of two ways. Feeding the output from the B & K amplifier into the oscilloscope together with the stimulus signal and displaying the Lissajous figures was one possibility. In the other more accurate method a phase meter was employed. It measured the phase difference between the stimulus and the output signal. The meter was built in this laboratory according to a circuit diagram published by Woodbury (1961). The accuracy of the meter was ±2 over the frequency range used in the experiments.

For the graphical representation of the CM patterns the phase of the most basal electrode was taken as zero reference phase. This was achieved simply by subtracting from all twelve phase readings the value measured for the most basal electrode.

An alternative method was adopted when it became necessary to measure the CM response at low SPLs (40 to 60 db). The output signals were too small and the noise in the recording system was too high for the previously described methods to work satisfactorily. The recovery of the waveform of the output signal from noise was accomplished with the aid of a digital computer PDP-9 situated in the computer room. The scheme for the computer aided measurements was devised by my colleague H. F. Ross, who also wrote the computer programme. The method was based on the technique known as signal averaging. The noisy signal was recurrently sampled at 50 successive points. The sampling points were so spaced in time as to cover one to two periods of the signal waveform. Sampling was initiated by pulses derived from the zero-crossings of the reference signal which was provided by oscillator O_1. Corresponding samples from recurrent sweeps were added up thus reinforcing the periodic signal waveform at each repetition. The signal waveform was displayed on the oscilloscope screen of the computer. From there it was transmitted via a television link back to the laboratory. In this way it was possible to observe the building up of the waveform of the output signal on the television screen during tonal stimulation. The stimulus to the animal was turned off (by opening switch S_1) as soon as the waveform of the output showed a satisfactory sinusoidal shape of sufficient amplitude. For any given stimulus

21	30	14 50				
1	0.1682E 02	0.1785E 02	0.2384E 02	0.1372E 01	0 0	316.4
2	0.1317E 02	0.1577E+02	0.2055E 02	0.1313E 01	3 7	320 1
3	0.7741E 01	0.1509E 02	0.1696E+02	0.1229E 01	16.4	332 8
4	-0.3311E+01	0.1318E 02	0.1351E+02	0.1131E 01	29 4	345.8
5	-0.4675E 00	0.1005E 02	0.1006E+02	0.1003E 01	39 8	356.2
6	0.1949E 01	0.2676E+01	0.3310E 01	0.5198E 00	7 5	323 9
7	-0.3370E+01	0.3518E 00	0.3415E 01	0.5334E 00	55 7	260 7
8	-0.5630E 01	0.1517E+01	0.5831E+01	0.7657E 00	61 5	254 9
9	0.6969E 01	0.1881E 01	0.7048E+01	0.8476E+00	38 2	278 2
10	0.5777E 01	0.2474E+01	0.6284E 01	0.7982E 00	23 2	293 2
11	0.3088E 01	0.2852E 01	0.3758E 01	0.5750E 00	-9 6	306.8
12	0.1992E 01	0.1955E 01	0.2791E 01	0.4456E 00	1 9	314.5

Fig. 2.9 Example of a computer print out. (The data are drawn in Fig. 3.1.A, see 14.5 kHz record).
Key: Row 1 Record 21 –30 db (corresponds to 85 db SPL), 14.50 kHz. Column 1 electrode numbers 1 to 12; 2 sine (electrode output); 3 cosine (electrode output); 4 CM amplitude in μV rms, 5 $\log_{10}$ CM amplitude 6, CM phase referred to phase value of electrode 1, 7 CM phase measured against stimulus signal.

all twelve electrode outputs were recovered in this way Then the computer data were stored on magnetic tape

This technique allowed the rapid collection of data. During a session of three to four hours the responses due to about 80 different stimuli could be measured. In terms of the previously employed techniques this meant taking 80 × 12 × 2 = 1 920 different accurate amplitude and phase readings—quite an impossible task even if there had not been any noise.

After the data collecting phase sinusoidal curves were fitted to the data stored on the magnetic tape and subsequently the computer printed out amplitude and phase values. (Here also the phase difference was measured between the reference signal (stimulus) and the output signal.) An example of such a print-out is given in Fig. 2.9

Since the cables leading from the laboratory to the computer room were quite long there was cross-talk from the reference channel) to the signal channel. The 'ghost signal' in the signal channel due to cross-talk had a magnitude corresponding to a CM amplitude of 0.05 to 0.5 μV rms. In cases where the CM had a value in the region of 2 to 5 μV rms the ghost signal was measured separately and later subtracted from the compound signal. The ghost signal was determined by measuring the response in the idle signal channel, i.e. with no CM signal applied.

DISCUSSION OF TECHNIQUE

It is expedient to discuss at this point some questions relevant to the application of the multi-electrode technique. There are basically two aspects which need consideration. The one aspect is concerned with the question whether the mechanical performance of the cochlear system is disturbed due to the slit opening and the insertion of the probe into scala tympani. The other aspect relates to the problem whether the mechanical presence of the array in the scala distorts the potential field set up by hair-cell activity

According to Johnstone & Boyle (1967) the fluid level in scala tympani has negligible influence on the mechanical response of the basal part of the basilar membrane thus there was no difference in their results whether the perilymph was present or absent in the basal scala tympani (provided the basilar membrane was not dried out. Johnstone 1969). This is consistent with von Békésy's observations (1960 *a*, p. 439) on dimensional models where the emptying of 'scala tympani' did not alter the position of the vibratory pattern manifested by the local stability of the circular eddy in 'scala vestibuli. Further evidence for the negligible effect of slit-like openings in the cochlear wall on the excitation pattern comes from the experiments by Tasaki et al. (1952). These authors made a small opening in the

wall of scala vestibuli in the second turn of the guinea pig s cochlea and they monitored the CM response in the third turn before and after the manufacture of the hole Due to the hole the CM response dropped only by an amount of 3 to 4 db (Tasaki et al came to the very interesting conclusion that a considerable portion of the acoustic energy must be transmitted to the upper parts of the cochlea by means of longitudinal coupling in the mem brane This is important since mathematical models of cochlear hydrodynamics usually neg lect coupling forces between the transverse ele ments of the basilar membrane (see Zwislocki, 1953) It is difficult to judge whether the hole produced a complete release of the pressure wave in the fluid column of scala vestibuli and therefore prevented energy transport via the fluid medium to the regions apical to the hole. If this was the case the conclusion of Tasaki et al. would be necessary Despite the fact that the hole had an area almost as large as the canal cross section it would probably be more realistic to visualize the effect of the hole as that of a hydraulic filter' with fre quency dependent characteristics. On the basis of this assumption one would predict the oc currence of a large attenuation of the apical signal in cases where the wavelength of the pressure wave approaches the dimensions of the hole i e at high frequencies where the wavelength is sufficiently short. Tasaki et al. reported that 'for all the frequencies' employed the drop in potential in the third turn was the same However in the case of a hole (width 200 μ) connecting the basal scala vestibuli with the scala tympani of the second turn they recorded an equal change of the apical poten tial 'for frequencies below 800 Hz this we may assume could mean that at higher fre quencies apical potentials were relatively more affected. The problem of which medium—the membrane or the fluid—is mainly responsible for the energy transport is extremely difficult to solve with experiments like those of Tasaki et al. Not only can an opening influence the hydrodynamics of the system but it can also influence the recording situation, due to the outflow of perilymph from the opening there exists the possibility of electrical cross-con nections between channels via the electrode holes. Clearly the necessity to keep these two effects separate seriously narrows the range of possible spatial configurations of electrode positions and opening positions.)

Evidence for the negligible influence of the slit in the wall of the basal scala tympani on the functioning of the cochlea is provided by the present study Fig 2.10 shows the CM amplitude patterns obtained from two different guinea pigs for the same stimulus parameters. The one curve was measured with the single electrode technique i.e. ten electrodes were essentially sealed into the holes in the wall of scala tympani The other curve was recorded with the multi-electrode technique, i e. the electrode array was inserted through the slit into scala tympani It can be seen that both techniques produced the same result.

The weight of the evidence mentioned so far seems to support the view that the effect of the opening in the basal part of scala tympani can be neglected. However there is a statement in the literature (von Békésy & Rosenblith, 1951 p 1105 von Békésy 1960 b p. 92) that a circular eddy occurs at any opening in the cochlear wall under strong tonal stimulation. It is stated further that covering the opening with a glass plate and immersing the whole preparation in fluid reduces the eddy to a great extent. In our experiments a considerable amount of perilymph was allowed to collect above the slit opening. Whether this fluid column above scala tym pani had an effect similar to that of the meas ures taken by von Békésy is, of course impos sible to decide on the basis of CM recordings.

A further point concerns the fact that the array tip was inserted into scala tympani so that a mechanical object was present in the tympanic channel. This situation is similar to some extent to the model study of von Békésy (1960 a p 437) where a small object was placed in scala tympani' Von Békésy observed

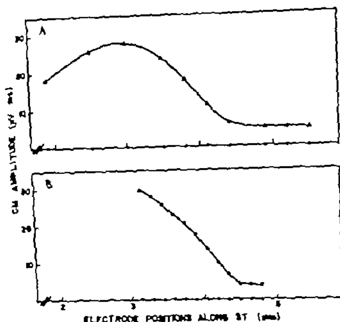


Fig. 2.10. CM amplitude pattern at 12 kHz, 90 db SPL, measured in two different *guinea pigs*. (Electrode position along scala tympani, *ST* is given.)

(*A*) Pattern obtained with the 'single electrode' technique (ten different electrodes).

(*B*) Pattern obtained with the 'multi-electrode' technique. The two different techniques gave the same results over the region studied.

a distortion of the eddy in scala tympani however the eddy in 'scala vestibuli' remained unchanged. This effect seemed to be related to the narrowing of 'scala tympani' due to the presence of the object and von Békésy concluded 'that nature had made the depth of the duct as small as possible without going so far as to cause a spreading out of the vibratory pattern along the cochlear partition. Since in our experiments there was *sufficient free space* between the actual array *and* the borders of the slit *one would assume that the* array did *not effectively narrow* the canal.

The other major question which needs consideration is concerned with the influence the mechanical presence of the array might have on the potential field in the scala. As shown in Fig. 2.10, both techniques employed in the study with their very different electrode arrangements gave the same results, hence it seems very unlikely that the array *probe* significantly distorts the potential field. In Fig. 2.11 A. the results from another *test experi*ment are displayed. The CM potentials were measured for the same *stimulus* at *two* different depths of insertion of the probe into scala tympani. Thus the length of the array tip inserted in the scala was different for the two measurements. There are only very slight differences between the patterns acquired from different depths and we may assume that these differences are due to the conductive *proper*ties of the inner ear rather than *the different* lengths with which the array projects into the scala. *Yet another piece of evidence* is illustrated in Fig. 2.11 B. Here the CM pattern of an 8 kHz tone was registered. (The animal was exposed previously to a traumatic tone of 13 kHz; this explains the peculiar shape of the pattern. See Section 3. Tonal Overstimulation.) This pattern was chosen *for the test experi*ment because there was a large *phase shift* between electrodes 5 *and* 6. The array was translated by a *small amount* in the longitudinal direction and the CM response to the same *stimulus* was measured again. As shown in the illustration we obtained essentially the same pattern, displaced however in the longitudinal direction. The large phase shift occurs now between electrodes 3 and 4. This is convincing evidence that the electrode probe itself has practically no influence on the results and can be regarded as a 'passive' sensing device meeting the purpose for which it was designed.

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3 Results

pothesis to be tested by the experiments es the cochlear microphonic distribution he cochlear ducts as the spatially filtered of the hair-cell activity along the membrane.

$$) = \int_{-\infty}^{\infty} H(z)\, W(x-z)\, dz = H(x) * W(x) \quad (1)$$

ion (1) shows the connection between and CM(x) for any arbitrary auditory us. In our experiments sinusoidal stimuli used and we may adopt the trigonometric e alternative exponential notation to y $H(x)$

usoidal stimulation of the inner ear s travelling wave motion along the coch- partition. The mechanical excursions of artition activate the hair-cell generators. nay represent the electrical output of any unit generator situated at a point x_a the basilar membrane in terms of a ier series. For an external stimulus of an frequency w this series takes the form.

$$, t) = A_0(x_a) + \sum A\ (x_a) \cos (nwt \quad B_n(x_a)) \quad (2a)$$

expression (2 a) indicates that the electri- output at x_a may contain harmonic com- ents due to nonlinearities in the transducer em. Since the space variable x assumes all ies x_a along the basilar membrane $h(x, t)$ otes the spatial distribution of the electrical put along the membrane:

$$, t) = A_0(x) + \sum A_n(x) \cos (nwt - B_n(x)) \quad (2b)$$

when written in exponential form

$$h(x\ t) = A_0(x) + \sum^{\infty} \mathrm{Re}[A_n(x)\, e^{\ jB_n(x)}\, e^{jnwt}] \quad (2c)$$

We obtain the instantaneous distribution of electrical potentials along the cochlear scalae $cm(x\ t)$ by spatially filtering $h(x\ t)$:

$$cm(x, t) = \int_{-\infty}^{\infty} [A_0(z) + \sum_1^{\infty} \mathrm{Re} \times (A\ (z)\, e^{\ jB_n(z)}\, e^{jnwt})]\, W(x-z)\, dz \quad (3a)$$

The time-dependent factors in (3 a) are unaf fected by the spatial integration and we can write

$$cm(x, t) = \int_{-\infty}^{\infty} A_0(\ )\, W(x-z)\, dz + \sum_1^{\infty} \mathrm{Re} \left[e^{jnwt} \int_{-\infty}^{\infty} A\ (\ )\, e^{\ jB_n(z)}\, W(x-\ )\, dz \right]$$

with

$$\int_{-\infty}^{\infty} A_0(z)\, W(x-z)\, dz = Q_0(x)$$

$$\int_{-\infty}^{\infty} A_n(z)\, e^{\ jB_n(z)}\, W(x-z)\, dz = Q_n(x)\, e^{\ jP_n(x)}$$

it follows

$$cm(x, t) = Q_0(x) + \sum_1^{\infty} \mathrm{Re}[Q_n(x)\, e^{\ jP_n(x)}\, e^{jnwt}]$$

$$cm(x, t) = Q_0(x) + \sum^{\infty} Q_n(x) \cos (nwt - P_n(x)) \quad (3b)$$

With a set of sensing electrodes placed along the cochlear duct the potential distribution $cm(x\ t)$ can be recorded. By extracting the fundamental frequency of the electrode out puts we obtain the distribution of the funda- mental component.

$$cm(x, t) = Q_1(x) \cos (wt - P_1(x)) \quad (4)$$

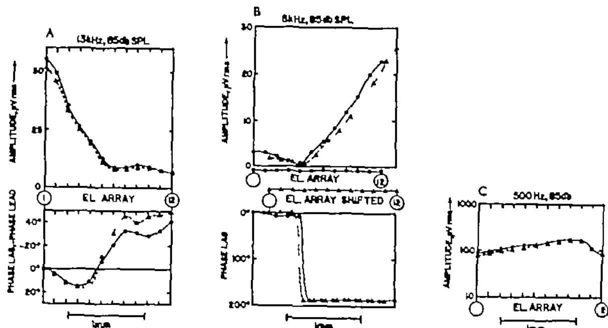


Fig 2 11 (A) CM amplitude and phase pattern at 13 kHz, 85 db SPL. The array was inserted to a depth of 400 μ (●—●) and 300 μ (▲—▲) into scala tympani. There is very little difference between the CM patterns measured at different depths.

(B) CM amplitude and phase pattern at 8 kHz, 85 db SPL (after overstimulation with a tone of 13 kHz, 130 db for 3 min). The electrode array was translated in the apical direction by approximately 700 μ and essentially the same CM pattern was obtained. The large phase shift between electrodes 5 and 6 occurred after the translation between electrodes 3 and 4 ●—● Pattern before translation ▲—▲ pattern after translation.

(C) CM amplitude patterns obtained from two different guinea pigs for the same stimulus (500 Hz, 85 db SPL). The two curves are almost identical ●—● first guinea pig. ▲—▲ second guinea pig. In (A) (B), (C) electrodes are numbered from 1 to 12 beginning with the most basal electrode.

Additional support for the validity of the CM recordings taken with the electrode array derives from the fact that each of the twelve electrode outputs had exactly the same properties as the CM recorded in the normal way by other workers with only one or two electrodes in the basal turn This concerns the magnitude of the potentials and the intensity function It suggests that the slit technique does not disturb the system more than the traditional technique with only a few small holes.

Theoretically speaking the multi-electrode technique cannot itself provide the answer to the question whether it causes disturbance in the cochlear system since such an answer requires the knowledge of the CM distribution in the completely undisturbed cochlea for comparison The evidence presented above strongly suggests a negligible interference of the technique with the functions of the cochlea but even if there was interference the technique would still be useful provided the interference was stable from experiment to experiment The question of reproducibility of results was one of major concern in this study since any uncontrolled variability in the CM patterns would make the quantitative investigation of the spatial filter effect completely impossible. The great pains taken both with the surgery and the equipment to set up the experiment proved worth while in the long run In Fig 2.11 C two CM amplitude patterns from different guinea pigs are shown for the same stimulus parameters It can be seen that the curves are almost identical Thus experiments yielded reproducible data.

a feature observed in the majority of the preparations. The drop in potential is probably an artefact due to the strong curvature of the basilar membrane and the scala tympani in this region. Electrode tips are arranged in the array in a straight line which approximates to the curved course of the cochlear partition and cochlear canals over a short distance only. In terms of the array configuration the cochlear canals take a sharp bend away from the array in the region of electrodes 11 and 12. Thus 11 and 12 are the electrodes dislocated most from the 'ideal' line following the longitudinal course of the membrane. It can be seen in the 500 Hz record that there is practically no alteration in the phase pattern at 11 and 12. This also indicates that the drop in amplitude at 11 and 12 is due to the placement of the array rather than phase cancellation. This conclusion results from the fact that in all high frequency records—where phase cancellation is manifest—amplitude variations are always accompanied by marked phase variations.

The goodness of a preparation may be judged on the basis of low frequency records because the expected patterns are simple and deviations from the normal response stand out clearly enough to allow rapid judgement. However, with the experience accumulated over many experiments it was possible to estimate the quality of preparations by considering the CM pattern at any frequency between 500 Hz and 15 kHz.

CM patterns take a more complicated form as the stimulus frequency is increased. The maximum of the excitation pattern along the membrane moves progressively towards the basal end of the cochlea with increasing frequency, the wavelengths of the excitation pattern become progressively shorter and this means that the phase lag gradient (phase change per unit length) along the partition increases with frequency. Closely neighbouring hair-cells are driven out of phase and consequently there will be negative interference, mutual cancellation of hair-cell outputs in the surrounding liquid spaces. The total number of hair-cells activated is probably smaller at high frequencies than at low ones because the excitation pattern is confined to a relatively shorter length of membrane. The general tendency of the CM amplitude to decrease when the stimulus frequency is raised can be read from Fig. 3.3.A. This tendency is probably due to the combined effect of the reduction in the number of activated hair-cells and the growing influence of phase cancellation with increasing frequency.

The CM amplitude distributions undergo characteristic changes with increasing stimulus frequency. Electrodes in the distant part of the array show relatively more drop in potential than do the proximal electrodes. The low level of the distant amplitudes is maintained when 15 kHz is exceeded and this observation indicates that the bulk of the excitation occurs in the region basal to this part of the array, that is at frequencies beyond 15 kHz. The most interesting feature, however, is the occurrence of a distinct amplitude minimum, well shown in the records at frequencies around 14 kHz.

The CM phase gradient along the array increases progressively with stimulus frequency (as shown in the records from 500 Hz to 12 kHz), thus at 12 kHz there is an overall phase difference between electrodes 1 and 12 of 196°. In the 13.5 kHz record this phase difference amounts to 258°, however the shape of the CM phase distribution is clearly different from that in the 12 kHz record. The phase gradient is small in the proximal and distal parts of the array while it is large in the region of the amplitude minimum. The co-occurrence of an amplitude minimum and a large phase gradient is a characteristic property of CM patterns at high frequencies, in the present example at frequencies around 13.5 kHz.

At 13.75 kHz the phase gradient in the region of the amplitude minimum is even greater than at 13.5 kHz. The phase difference between electrodes 6 and 7 has a value of 143°. Clearly this is an enormous local varia-

The amplitude distribution $Q_1(x)$ and the phase distribution $P_1(x)$ define the spatial distribution of the fundamental component of the cochlear microphonic. Both parameter distributions can be determined experimentally by measuring the amplitude and phase values of all electrode outputs.

Since we are only interested in the amplitude and phase distributions it is preferable to use the exponential instead of the trigonometric notation It is permissible to omit the time variable because it does not enter calculations. It therefore suffices to write

$$CM_1(x) = \int_{-\infty}^{\infty} H_1(x)\, W(x-z)\, dz = H_1(x) * W(x) \quad (5)$$

$$CM_1(x) = Q_1(x)\, e^{\,jP_1(x)} \quad (5\,a)$$

$$H_1(x) = A_1(x)\, e^{\,jB_1(x)} \quad (5\,b)$$

The expressions in (5 *a*) and 5 *b*) show the essential parameter distributions involved in the *spatial filtering operation* The amplitude and phase distributions of the cochlear microphonic are related to the amplitude and phase distributions of the hair-cell outputs according to equation (5) Multiplication of equation (5) with a constant reference phase factor exp(jP) has no influence on the spatial integration. It is therefore permissible to refer the phase readings of the electrode outputs to any arbitrary phase value In the following diagrams the output phases are always referred to the phase value of the most basal electrode. Since in our experiments we exclusively considered the fundamental component of the cochlear microphonic we may simplify the notation in the text. Thus, with $H(x)$ we mean the spatial distribution of the fundamental component of the hair-cell outputs in response to sinusoidal stimuli $CM(x)$ or $CM(x_e)$ denotes the longitudinal distribution of the fundamental component of the cochlear microphonic in response to sinusoidal stimuli

All CM patterns displayed in this section were obtained with the multi-electrode array The most basal electrode of the array was always located in the region of 3 to 3.5 mm, measuring from the basal end of the basilar membrane. In the graphs the space coordinate is indicated by the electrode positions (see Fig 3 1.B) It is expedient for the discussion of the CM patterns to express the space coordinate in terms of electrode numbers. Thus the electrodes of the array are numbered from **1** to **12** beginning with the most basal electrode

A. THE DESCRIPTION OF DATA

CM amplitude and phase patterns

Experiments were carried out by measuring the amplitude and phase distribution of the CM along the electrode array for tonal stimuli of different frequencies. In all experiments we obtained response patterns with the same basic properties, all characteristic features of the distributions being reproducible in different guinea pigs.

Fig. 3 1.A shows a typical example of a set of CM patterns from one guinea pig the patterns were measured at frequencies from 500 Hz to 15.5 kHz and a constant SPL of 85 db The low frequency (500 Hz) record can be used as a control of the preparation and the uniformity of the sensing electrodes. At this frequency all individual electrodes composing the array are essentially equidistant from the maximum excitation which occurs in the apical portion of the cochlea. The wave length in the basal part of the excitation pattern is very long compared to the array width and we may therefore expect very little phase variation along the array This expectation is confirmed by the 500 Hz record which shows negligible phase change between electrodes Due to the long wavelength of the excitation pattern there is practically no phase cancellation in the region of the array and consequently the CM amplitude pattern has an appearance similar to that of the basal part of the envelope of the excitation pattern. Thus the amplitude in the 500 Hz pattern grows continuously in the apical direction However at electrodes **11** and **12** the amplitude declines,

a feature observed in the majority of the preparations. The drop in potential is probably an artefact due to the strong curvature of the basilar membrane and the scala tympani in this region. Electrode tips are arranged in the array in a straight line which approximates to the curved course of the cochlear partition and cochlear canals over a short distance only. In terms of the array configuration the cochlear canals take a sharp bend away from the array in the region of electrodes 11 and 12. Thus 11 and 12 are the electrodes dislocated most from the 'ideal' line following the longitudinal course of the membrane. It can be seen in the 500 Hz record that there is practically no alteration in the phase pattern at 11 and 12. This also indicates that the drop in amplitude at 11 and 12 is due to the placement of the array rather than phase cancellation. This conclusion results from the fact that in all high frequency records—where phase cancellation is manifest—amplitude variations are always accompanied by marked phase variations.

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The CM amplitude distributions undergo characteristic changes with increasing stimulus frequency. Electrodes in the distant part of the array show relatively more drop in potential than do the proximal electrodes. The low level of the distant amplitudes is maintained when 15 kHz is exceeded and this observation indicates that the bulk of the excitation occurs in the region basal to this part of the array that is at frequencies beyond 15 kHz. The most interesting feature, however is the occurrence of a distinct amplitude minimum, well shown in the records at frequencies around 14 kHz.

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At 13.75 kHz the phase gradient in the region of the amplitude minimum is even greater than at 13.5 kHz. The phase difference between electrodes 6 and 7 has a value of 143 Clearly this is an enormous local varia-

tion considering that the interelectrode distance is only 150 μ. The appearance of the phase pattern changes abruptly when the stimulus frequency exceeds 13 75 kHz. Thus at 14 kHz the distant part of the array definitely leads the proximal part in phase. This phase reversal in the CM pattern at high frequencies can only be observed by means of closely spaced electrodes. The technique of Tasaki et al. (1952) with only two recording points in the basal coil of the cochlea is completely inadequate for the study of the CM phase variation at high frequencies. The single electrode technique described in Section 2, with a minimum distance of 300 μ between electrodes was not good enough for the recording of complicated phase patterns like those displayed in the records from 14 kHz to 15 5 kHz. Comparison between the phase patterns at 15 kHz and at 15.5 kHz reveals a distinct shift of the zero-crossings, maxima and minima of the 15.5 kHz phase patterns by about one electrode distance in the basal direction The overall phase variation along the array decreases noticeably from 14.5 kHz to 15 5 kHz.

We may summarize those features of the CM patterns which are most important for the demonstration of the spatial filter effect. 1) Amplitude minima are always accompanied by large changes in phase between adjacent electrodes. 2) Phase reversal occurs at high stimulus frequencies and distant electrodes may lead proximal ones in phase

CM patterns in vector form

CM patterns represent complex functions of the real space variable x since they comprise two parameter distributions, those of amplitude and phase The two distributions may be plotted separately as shown in Fig 3 1 A, or they may be combined in a single 3-dimensional graph, with the dimensions of the graph given by a set of complex planes arranged along the space coordinate x This is because an electrode output at x can be visualized as a complex number or a vector in the complex plane at x with the amplitude and phase value of the electrode output representing the vector length and vector phase angle respectively Thus it is easy to imagine the CM distribution as a line connecting the endpoints of the output vectors in the complex planes at the electrode positions 1 to 12. However for the purposes of the present discussion it is sufficient to consider the projection of this line into a single complex plane, say the plane at electrode 1 We may conveniently call the projected version of the CM distribution the 'vector representation' or 'vector form' It is constructed by simply plotting all twelve output vectors in the same plane and by joining the vector endpoints through a trajectory (For completeness of the definition it may be mentioned that this trajectory denotes the projection of the complex CM distribution into a single plane.)

In Fig 3 2.A, B C the data from Fig 3 1 A are redrawn in vector form for the stimulus frequencies 13.5 13 75 and 14 kHz. Phase reversal occurs in this frequency region and the vectorial representation of data is best suited to illustrate this phenomenon. At 13.5 kHz the CM pattern exhibits progressive phase lag along the array from 1 to 12. By measuring the phase lag in the clockwise direction we obtain for the 13.5 kHz record a trajectory which encircles the origin of the vector plane Clearly any trajectory characterised by a progressive phase lag encircles the origin With increasing stimulus frequency the trajectory approaches and eventually crosses the origin After crossing phase reversal within the vector bundle must occur because the trajectory no longer encircles the origin. Thus in the 14 kHz record there is no encirclement of the origin, and it can be seen that the phase reverses between electrodes 5 and 6 electrode 6 leading in phase electrode 5

Vector representation of the data discloses the occurrence of the phase reversal with increasing frequency as the result of a simple continuous process. This is due to the fact that in this representation the CM amplitude

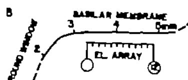


Fig. 3.1. (A) Typical example of a set of CM amplitude and phase patterns measured in one guinea pig. Note the co-occurrence of an amplitude minimum and large phase gradient in the 13.5 kHz and 13.75 kHz record. CM phase patterns beyond 13.75 kHz show phase reversal.

(B) Position of electrode array (*EL. ARRAY*) in relation to the length coordinate of the basilar membrane. —— amplitude; – – – phase.

and phase data are considered in combined vector form. If the phase pattern is considered alone, like in Fig. 3.1.A, the transition from the 13.75 kHz to the 14 kHz phase pattern appears to be an abrupt event, and therefore quite irritating to the observer.

A simple model can be given to explain the transition from a trajectory with progressive

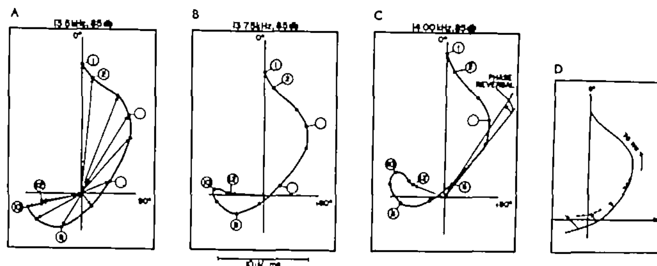


Fig 3.2 (A–C) Records (13.5 kHz, 13 75 kHz, 14 kHz) from Fig. 3 1.A redrawn in vector form. In (A) all output vectors are shown in (B) and (C) all vectors apart from 1 and 12 are omitted for clarity The trajectory connects all vector endpoints. Phase lag is counted in the clockwise direction The trajectory approaches with increasing frequency the origin of the vector plane note the concomitant increase in phase difference between 6 and 7 In (C) phase reversal occurs, 6 leading 5 in phase. In (A) and (B) the trajectory encircles the origin In (C) it does not.

(D) Artificially induced phase reversal by addition of small vector with large phase lag and amplitude which declines as the round window (*RW*) end of the array is approached.

phase lag (13 75 kHz) into one with phase reversal (14 kHz) This is illustrated in the schematic drawing of Fig 3 2.D Let us add to all electrode outputs in a pattern like the one at 13 75 kHz a small vector with a large phase lag and a length which declines as the basal end of the array is approached. The resulting trajectory has an appearance like the one at 14 kHz, i e it does not encircle the origin Something analogous to this vector addition may be expected to happen in reality As the stimulus frequency is increased from 13 75 to 14 kHz the wavelengths in the excitation pattern will shorten and a small group of distant hair-cells will be operating with slightly larger phase lag than before The influence of these distant hair-cell outputs is of course felt with an amplitude which declines towards the basal end of the array The model provides a qualitative explanation for the observed phase reversal and it suggests the far reaching influence of alterations in the outputs of subsets of hair-cell operators on the appearance of the whole CM pattern an influence postulated by the spatial filter hypothesis.

A further point which deserves to be mentioned concerns the situation where the trajectory actually crosses the origin This situation was thoroughly explored in a preparation where one electrode of the array recorded very nearly zero amplitude in the frequency region of the phase reversal Thus this electrode output, when plotted on the trajectory very nearly coincided with the origin of the vector plane. The output of this electrode was displayed on the oscilloscope screen and a faint sinusoidal signal was just noticeable in the background noise It was interesting to observe that the output signal was extremely unstable shifting abruptly back and forth by about 180 on the oscilloscope screen All other electrode outputs, however were perfectly stable This finding, although it may seem strange at first glance can be interpreted quite plausibly Consideration of Fig 3 2 shows that the phase difference between the adjacent electrodes 6 and 7 grows continuously when the trajectory approaches the origin When the trajectory crosses the origin this phase difference becomes approximately 180 Thus a point in the immediate vicinity of the origin, intermediate to 6 and 7 is framed by two vector bundles ap-

proximately 180° out of phase with each other. This situation may be visualized as an unstable equilibrium, any small disturbance causing the small intermediate vector to shift to either of the two bundles.

B THE ANALYSIS OF DATA

The spatial filter hypothesis is stated in the form of the convolution integral in equation (1):

$$CM(x) = H(x) * W(x) \tag{1}$$

For the demonstration of the spatial filter effect the following mathematical procedure was adopted. A certain hypothetical function $H(x)$ was assumed and subjected to the convolution in equation (1). The shape of $H(x)$ was chosen so as to resemble excitation patterns likely to exist in reality. The resulting hypothetical $CM(x)$ distribution was examined for properties similar to the ones found in the experimental data. In the event that a hypothetical $CM(x)$ closely approximated to a given real CM pattern it was further assumed that the corresponding hypothetical $H(x)$ approximated to the real hair-cell output pattern along the basilar membrane.

However, according to equation (1) $CM(x)$ depends on $H(x)$ and the weighting function $W(x)$ and consequently it is important to use in the mathematical analysis a function $W(x)$ based on experimental observations. Fortunately useful data about $W(x)$ are available in the literature.

The weighting function $W(x)$

The electrical attenuation properties of the cochlear ducts in the guinea pig were studied by four different investigators or groups of investigators (von Békésy, 1960 *a*; Tasaki & Fernández, 1952; Tasaki et al., 1952; Misrahy et al., 1958; Johnstone et al., 1966). All investigators agree that the longitudinal spread of current in the basal turn can be accurately described by a core conductor model. In the apical parts of the cochlea complication arises due to the close packing of turns and possible alternative paths of cross-conduction between turns. Since in our experiments data were collected in the basal turn we only need to consider conditions there.

Von Békésy thoroughly investigated the spread of signals along the basal part of the cochlear partition over a length of 9 mm beginning at a distance of 0.5 mm from the basal end of the membrane. He used a set of paired electrodes placed oppositely in scala tympani and scala vestibuli along the first turn. A test signal was injected across the partition at one pair of electrodes and the resulting attenuated signal was measured at the other sensing electrode pairs. The signal attenuation he obtained amounted to 6 db per mm. Moreover, he observed that there was no 'remarkable change in the attenuation along the partition whether measured in the apical or basal direction. Thus the signal attenuation for a generator pair of electrodes situated at a position x along the partition is symmetrical with respect to x. Von Békésy employed signals with frequencies ranging from 100 Hz to 2 kHz and he could not find any dependence of the attenuation value on signal frequency. This indicates that the signal attenuation along the partition is predominantly due to the resistive properties of tissues and fluids and that the capacitive properties of the structures have negligible influence. Tasaki & Fernández applied a d.c. signal across the partition in the basal turn and they also measured a decay of signal from the site of application of 6 db per mm. Von Békésy's extensive study well established the core conductor type of the electrical attenuation along the partition and we can formulate mathematically the attenuation or weighting function.

$$W(x) = e^{-|x|/\theta} \tag{6}$$

x and θ in mm. θ denotes the length constant or decay constant of the longitudinal signal attenuation. In the core conductor model θ takes the form.

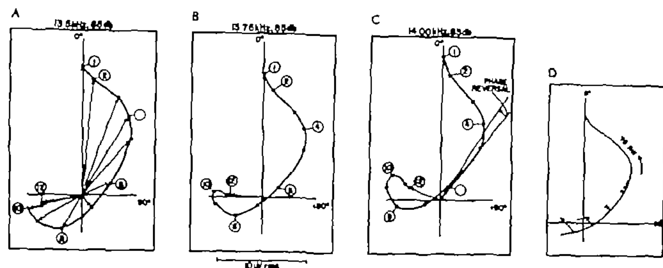


Fig 3.2 (A–C) Records (13.5 kHz, 13.75 kHz, 14 kHz) *from Fig. 3.1.A redrawn in vector form.* In (A) all output vectors are shown, in (B) and (C) all vectors apart from *1* and *12 are omitted for clarity* The trajectory connects all vector endpoints. Phase lag is counted in the clockwise direction The trajectory approaches with increasing frequency the origin of the vector plane note the concomitant increase in phase difference between *6* and *7* In (C) phase reversal occurs, *6* leading *5* in phase. In (A) and (B) the trajectory encircles the origin, in (C) it does not.

(D) Artificially induced phase reversal by addition of small vector with large phase lag and amplitude which declines as *the round window (RW) end of the* array is approached.

phase lag (13.75 kHz) into one with phase reversal (14 kHz) This is illustrated in the schematic drawing of Fig. 3.2.D Let us add to all electrode outputs in a pattern like the one at 13.75 kHz a small vector with a large phase lag and a length which declines as the basal end of the array is approached. The resulting trajectory has an appearance like the one at 14 kHz, i e it does not encircle the origin Something analogous to this vector addition may be expected to happen in reality As the stimulus frequency is increased from 13.75 to 14 kHz the wavelengths in the excitation pattern will shorten and a small group of distant *hair-cells will be operating* with slightly larger phase lag than before The influence of these distant hair-cell outputs is of course felt with an amplitude which declines towards the basal *end of the array The model provides a quali*tative explanation for the observed phase reversal and it suggests the far reaching influence of alterations in the outputs of sub*sets of hair-cell operators on the appearance* of the whole CM pattern, an influence postulated by the spatial filter hypothesis.

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cochlea. We have to adapt this pattern to high frequencies in order to make the distribution CM(x) resulting from the spatial filter operation comparable to our high frequency records. We achieved this adaption by compressing the 'von Békésy' pattern into a shorter length of membrane. With the further assumption that hair-cell output is directly proportional to the local mechanical stimulus we obtained a hypothetical hair-cell distribution $H(x)$.

With the functions $H(x)$ and $W(x)$ (see equation (6)) given we can execute the spatial filter operation. The convolution integral

$$CM(x_0) = \int_{-\infty}^{\infty} H(x)\exp(-0.69|x_0 - x|)\,dx$$

was solved by graphical integration.

Thus the above formula, continuous in x was substituted by

$$CM(x_0) = \frac{m-n}{s}\sum_{i=0}^{s} H(x_i)\exp(-0.69|x_0 - x_i|) \quad (8)$$

where

$$x_i = n + i\,\frac{m-n}{s} \qquad i = 0, 1, \ldots, s$$

and

$$x_i, x_0 \in [n, m]$$

In equation (8) the limits of the integration interval $[-\infty, \infty]$ are replaced by the finite boundaries n, m. This is permissible provided the bulk of the pattern $H(x)$ lies within the interval $[n, m]$. For the graphical integration the interval $[n, m]$ was subdivided in $s = 10$ equal intervals.

Each $H(x_i)$ in equation (8) is characterized by an amplitude and a phase value and can therefore be represented as a vector. Thus for each x_0 ($x_0 \in [n, m]$) $CM(x_0)$ forms the vector sum of the contributions $H(x_i)$ weighted by the appropriate attenuation factor. In Fig. 3.3 all functions involved in spatial filtering are shown. The graphical integration yields the pattern $CM(x_0)$ in vector form and it can be seen in the vector plot in (A) how the weighted vectors $H(x_i)$ add up to $CM(x_0)$ for $x_0 = n$ (see

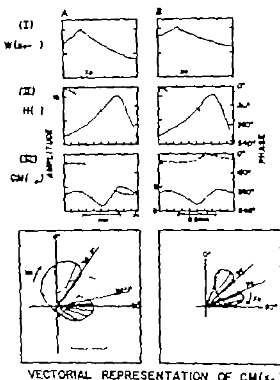


Fig. 3.3. Spatial Filtering of 'von Békésy' pattern $H(x)$. In (A) $H(\ )$ is compressed into interval $[n, m] = 2$ mm. In (B), analogous to increase in frequency $[n, m] = 1$ mm. All functions involved in spatial filtering are shown.

I. $W(x)$: weighting function, drawn for an arbitrary x_0. $W(x) = \exp(-0.69|x_0 - x|)$

II. $H(x)$: hypothetical hair-cell output distribution of the 'von Békésy type'

III. $CM(x_0)$: hypothetical CM distribution resulting from spatial filtering.

Bottom graph is the vector representation of $CM(x_0)$. The convolution integral was solved by graphical integration; $[n, m]$ was subdivided into $s = 10$ equal intervals:

$$CM(x_0) = \frac{m-n}{s}\sum_{i=0}^{s} H(x_i)\exp(-0.69|x_0 - x_i|);\ x_i, x_0 \in [n, m]$$

As an example it is shown in the vector plane in (A) how $CM(x_0)$ forms the vector sum of the weighted contributions $H(x_i)$ for $x_0 = n$ ( ). In the 'lower frequency' example (A) the trajectory encircles the origin of the vector plane, in the 'higher frequency' example (B) it does not. Note co-occurrence of amplitude minimum and large phase gradient in (A,III). ——, amplitude; – – –, phase.

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$$\theta = \frac{1}{\sqrt{dR\,dG}} \qquad (7)$$

where dR signifies the longitudinal resistance per unit length of the fluid filled canals and dG signifies the longitudinal conductance per unit length of the cochlear partition. Tasaki et al. measured the resistances involved and calculated (see equation (7) and (6)) the attenuation along the partition They obtained a value of 6 db per mm They measured an electrical impedance across the cochlear partition of 100 to 200 Ω per cm in the basal turn at a frequency of 500 Hz. At 6 kHz they found an impedance about 15% less. Since this variation in impedance with frequency is very small compared to the variation of impedance at a single frequency (500 Hz) we may consider the attenuation independent of frequency This implies, of course, that λ in equation (6) is considered a real quantity that is neglecting capacitive effects of intracochlear structures.

Data considered so far concerned the attenuation of signals across the cochlear partition. The result unanimously achieved, gives an attenuation of 6 db per mm, i.e a decay constant $\theta = 1.45$ mm ($\lambda = 0.69$) However it needs to be clarified whether this value of θ is a good approximation to the real situation where the signal is generated by the hair cells within the organ of Corti, presumably across the reticular lamina and not as in the above test cases across the cochlear partition It is likely that a pair of electrodes, the one electrode situated in scala media and the other in scala tympani, mimics the real situation better than does a pair of electrodes situated across the whole cochlear partition Experiments to explore the attenuation along the basilar membrane have been done with electrodes inserted into scala media and scala tympani

Misrahy et al measured the resistances involved in this latter situation and they calculated (using equation (7)) for the basal turn a decay constant $\theta = 1.18$ mm for the signal spread along the basilar membrane However Johnstone et al., measuring the spread of current pulses along the membrane with electrodes in scala media, obtained an average length constant $\theta = 2$ mm in the first turn The discrepancy between these results is probably due to the fact that these experiments are extremely difficult to execute requiring utmost surgical cleanliness a requirement not easy to meet when handling sets of recording electrodes. Taking the arithmetic mean of the two values gives a mean length constant $\theta = 1.59$ mm a value not significantly different from the length constant along the whole cochlear partition Thus for the mathematical operations in this thesis we used von Békésy's value for the length constant corresponding to a signal decay of 6 db per mm

The weighting function in equation (6) takes into account the longitudinal spread of signal along the cochlear scalae it does not take account of the signal variation across the scalae. In accordance with the core conductor model we may neglect as a first approximation the effect of the distance between electrode tips and the basilar membrane on the CM distribution In Fig 2 11 A the CM pattern for the same stimulus parameter is shown measured at two different depths of insertion of the electrode array into scala tympani thus the distance between electrode tips and the basilar membrane was slightly different in the two records. There are only minor differences between the two patterns and we may therefore regard the potential variation across the scala tympani negligible as a first approximation

Spatial filtering of hypothetical patterns $H(x)$

It is expedient to begin the mathematical analysis of the spatial filter effect by subjecting a function $H(x)$ to spatial filtering which has the shape of the mechanical excitation curves measured by von Békésy The highest stimulus frequency at which von Békésy observed the complete mechanical excitation pattern along the membrane was 300 Hz the pattern extended from about 19 mm to 29 mm along the basilar membrane of the human

cochlea. We have to adapt this pattern to high frequencies in order to make the distribution CM(x) resulting from the spatial filter operation comparable to our high frequency records. We achieved this adaption by compressing the 'von Békésy' pattern into a shorter length of membrane. With the further assumption that hair-cell output is directly proportional to the local mechanical stimulus we obtained a hypothetical hair-cell distribution $H(x)$.

With the functions $H(x)$ and $W(x)$ (see equation (6)) given we can execute the spatial filter operation. The convolution integral

$$CM(x_0) = \int_{-\infty}^{\infty} H(x) \exp(-0.69|x_0 - x|)\, dx$$

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Thus the above formula, continuous in x was substituted by

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where

$$x_i = \quad + i\frac{m}{s} \frac{n}{} \quad i = 0\ 1 \quad s$$

and

$x \ \ x_0 \in [n, m]$

In equation (8) the limits of the integration interval $[-\infty\ \ \infty]$ are replaced by the finite boundaries n, m. This is permissible provided the bulk of the pattern $H(x)$ lies within the interval $[n\ m]$. For the graphical integration the interval $[n\ \ m]$ was subdivided in $s = 10$ equal intervals.

Each $H(x_i)$ in equation (8) is characterized by an amplitude and a phase value and can therefore be represented as a vector. Thus for each x_0 ($x_0 \in [n\ m]$) CM(x_0) forms the vector sum of the contributions $H(x_i)$ weighted by the appropriate attenuation factor. In Fig. 3.3 all functions involved in spatial filtering are shown. The graphical integration yields the

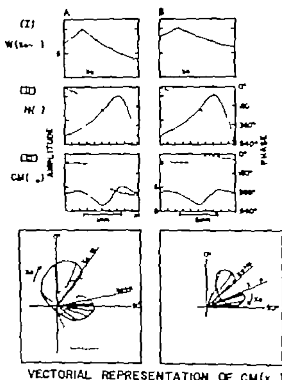


Fig. 3.3. Spatial Filtering of von Békésy pattern $H(\ )$. In (A) $H(x)$ is compressed into interval $[n, m] = 2$ mm. In (B), analogous to increase in frequency $[n, m] = 1$ mm. All functions involved in spatial filtering are shown.

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III. CM(x_0): hypothetical CM distribution resulting from spatial filtering.

Bottom graph is the vector representation of CM(x_0). The convolution integral was solved by graphical integration; $[n, m]$ was subdivided into $s = 10$ equal intervals.

$$CM(x_0) \quad \frac{m-n}{s} \sum_{i=0} H(x_i) \exp(-0.69|x_0 - x_i|); \quad x_0 \in [n, m]$$

As an example it is shown in the vector plane in (A) how CM(x_0) forms the vector sum of the weighted contributions $H(x_i)$ for x_0 = n ( ). In the 'lower frequency' example (A) the trajectory encircles the origin of the vector plane, in the 'higher frequency' example (B) it does not. Note co-occurrence of amplitude minimum and large phase gradient in (A,III). —— amplitude - - - - phase.

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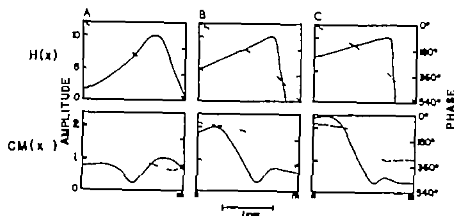


Fig 3.4 Modification of von Békésy type pattern $H(x)$. (A) $H(x)$ redrawn from Fig. 3.3 A. (B, C) Flattening the basal slope and steepening the apical slope of the envelope of $H(x)$ produces theoretical curves $CM(x)$ more similar to experimental CM patterns. —— amplitude - - - phase.

dashed line in vector diagram (A)) By plotting the amplitude and phase pattern of $CM(x_0)$ separately we obtain the hypothetical cochlear microphonic distribution $CM(x_0)$ in a fashion ready for comparison with experimental patterns.

In Fig. 3 3 A $H(x)$ was compressed into an interval length of 2 mm while in (B) analogous to an increase in frequency the interval length was only 1 mm Let us call A the 'lower frequency' and B the 'higher frequency' example

Practically all significant features of the resulting patterns $CM(x_0)$ are consistent with experimental findings. The amplitude of $CM(x_0)$ in the 'higher frequency' case is smaller than in the 'lower frequency' case this is compatible with real patterns which showed an overall drop in amplitude with increasing stimulus frequency The overall phase variation along the space coordinate in the 'higher frequency' example is very small a feature well shown by the experimental patterns in Fig 3 1 A at high stimulus frequencies. In the 'low frequency' example there is progressive phase lag along x_0 thus the trajectory in the vector representation encircles the origin of the vector plane In the high frequency example no encirclement of the origin occurs and the phase pattern shows phase reversal the more apical part of the pattern leading in phase the more basal part The occurrence of phase reversal with increase in stimulus frequency was one of the main characteristics of experimental CM patterns. In the 'lower frequency' example we observe the co-occurrence of an amplitude minimum and a large phase gradient. This also was a main characteristic of experimental CM patterns.

Thus it is demonstrated that all salient features of the measured CM patterns can be qualitatively reproduced by spatially filtering an excitation pattern of the von Békésy type It should be noted that $CM(x_0)$ at a particular point x_0 does not reflect the hair-cell output at this point x_0 this can be clearly seen in Fig. 3.3 Thus the notion held by several investigators that an electrode records local hair-cell activity is proved incorrect

Quantitative comparison between theoretical and experimental CM patterns

In the foregoing section we showed the excellent qualitative agreement between theoretical CM patterns deducible from the 'von Békésy type' function $H(x)$ and our own experimental data. Yet, closer inspection of the theoretical curves $CM(x_0)$ in Fig 3 3 reveals that the quantitative agreement with measured patterns is rather poor this applies especially to the shape of the amplitude pattern of $CM(x_0)$ which shows a large distant maximum.

We attempted to improve the quantitative fit between the theoretical and measured CM patterns by systematically modifying the shape of the hypothetical pattern $H(x)$. Since $H(x)$ consists of two parameter distributions, those of amplitude and phase, we can manipulate either or both of the two functions. Due to the lack

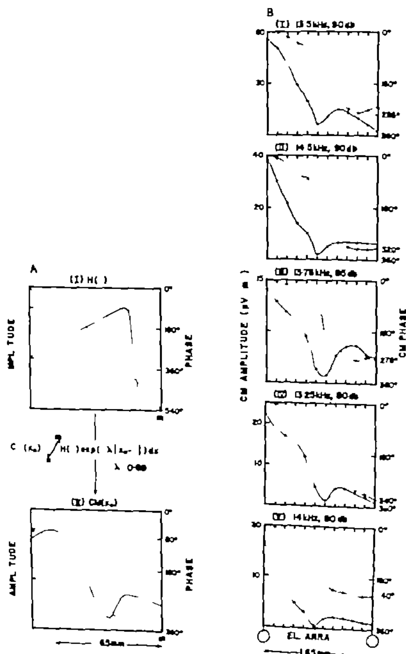


Fig 3.5 (A.I, II). Hypothetical $H(x)$ and $CM(x_0)$ re drawn from Fig. 3.4 B. Compare $CM(x_0)$ over the length 1.65 mm with experimental CM patterns in (B I–V)

(B I–V) Experimental CM amplitude and phase patterns obtained from five different guinea pigs. Stimulus parameters are essentially the same (stimulus frequency varied from 13.5 kHz to 14.5 kHz; SPL varied from 80 to 90 db) Note: Distinct amplitude minimum around 6 and 7, flat amplitude maximum around 8 and 9, large phase gradient in the region of amplitude minimum. On account of good quantitative agreement between $CM(x_0)$ (A.II) and experimental CM patterns (B.I–V) assume $H(x)$ in (A.I) to be a close approximation— $H(x)^*$—to the real hair cell output patterns for the stimuli in (B.I–V), i.e. for stimulus frequencies between 13.5 and 14.5 kHz. —— amplitude, - - - phase.

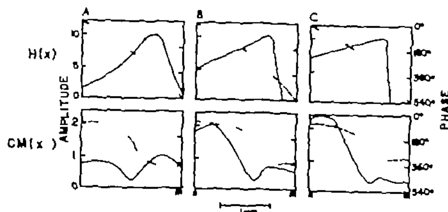


Fig 3.4 Modification of 'von Békésy type' pattern $H(x)$. (A) $H(x)$ redrawn from Fig. 3.3.A. (B, C) *Flattening the basal slope and steepening the apical slope of the envelope of $H(x)$ produces* theoretical curves $CM(x_0)$ more similar to experimental CM patterns. —— amplitude ---- phase.

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H(x) and the frequency response curve

We obtained from the considerations outlined in the previous section a distribution $H(x)^*$ representing the approximate shape of the hair cell activity pattern along the membrane at stimulus frequencies from 13.5 kHz to 14.5 kHz. We are now in the position to compare this information about the excitation pattern with the data available from mechanical measurements. However $^*H(x)$ is a distribution along the space variable x while all the mechanical data are frequency response curves of points along the membrane, i.e. they are functions of the frequency variable. For the comparison between the two sets of data we have to deduce from the shape of $H(x)$ the corresponding frequency response curve. This deduction can be done provided we know the conversion formula relating the space variable x to the stimulus frequency f in the guinea pig's cochlea.

Greenwood (1961) formulated the connection between stimulus frequency and the location of the maximum displacement along the basilar membrane in the guinea pig. He used the expression.

$$f = A(10^{ar} - 1) \qquad (9\,a)$$

where f denotes stimulus frequency in Hz and r denotes the distance in mm of the maximum displacement from the apical end of the membrane. In this thesis we measured the spatial coordinate x from the basal end of the membrane. In the guinea pig the basilar membrane has a total length of 18.5 mm. Thus by using the substitution

$$r = 18.5 - x$$

we can write equation (9 *a*) in the form.

$$f = A(10^{a(18.5-x)} - 1) \qquad (9\,b)$$

Greenwood determined the values (A, a) by fitting the function (9 *a*) to von Békésy's mechanical data measured in the upper turns of the cochlea. He obtained two sets of values, the one set giving rise to function G

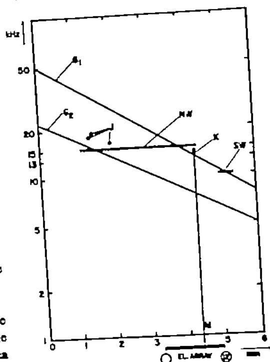


Fig. 3.7 Stimulus frequency on logarithmic scale plotted versus locus of maximum displacement along the guinea pig's basilar membrane. The space coordinate is measured from the basal end of the membrane; it is given in mm.

G_1 and G: Greenwood's functions.

SW: Damage of organ of Corti after overstimulation with 10 kHz tone, observed by Smith & Wever (1949).

NW: Region of swollen hair-cell nuclei after exposure to 15 kHz tone, observed by Neubert & Wustenfeld (1955).

$\vdash$: Frequency of maximum displacement for points along the guinea pig membrane, observed by Johnstone & Boyle (1967) and Johnstone (1969).

K: The position of the electrode array relative to the space coordinate is shown. M indicates the position of the envelope maximum of $H(x)^*$ according to Fig. 3.5. M falls into the region between electrodes 8 and 9. With M and the stimulus frequencies (13.5 kHz to 14.5 kHz) given for which $H(x)^*$ is considered an approximation to the hair-cell output distribution we can plot our results into the graph. They are marked K and they show good agreement with G.

$$G: \; f = 166.2(10^{[illegible](18.5-x)} - 1)$$

the other set leading to G_2.

$$G_2: \; f = 253(10^{[illegible](18.5-x)} - 1)$$

Both functions G and G_2 are shown in Fig. 3.7. The function G_2 fits the low frequency

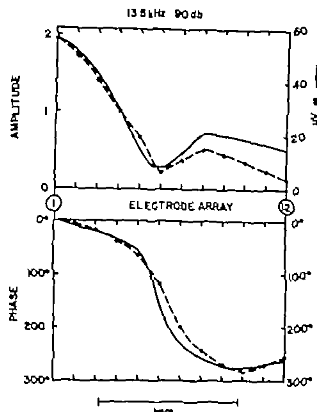


Fig. 3.6. Hypothetical CM(x_0) from Fig. 3.4 B and Fig. 3.5.A.II drawn together with experimental CM pattern from Fig. 3.5 B I. The relative amplitude for CM(x_0) is indicated on the left hand scale, the amplitude for the experimental 13.5 kHz, 90 db pattern is indicated on the right hand scale in μV rms. Both, amplitude and phase patterns show good quantitative agreement. —— hypothetical CM(x_0) – – – measured CM pattern at 13.5 kHz, 90 db.

of experimental data on the longitudinal phase variation in the mechanical excitation pattern at frequencies beyond 300 Hz we left the phase pattern of $H(x)$ unchanged. The amplitude envelope of $H(x)$ was modified and the corresponding CM(x_0) was calculated by graphical integration according to equation (8).

In Fig. 3.4 the result of this procedure is shown. Fig. 3.4 A displays the 'von Békésy type' pattern $H(x)$ used in Fig. 3.3 A. It can be seen that flattening the basal slope and steepening the apical slope of the envelope of $H(x)$ produces CM(x_0) patterns which approximate in shape very closely to experimental CM patterns. The CM(x) in Fig. 3.4 B gives the closest fit and it is redrawn in Fig. 3.5 A together with experimental CM distributions.

It can be seen at once that there is very good agreement between the hypothetical CM(x_0) in Fig. 3.5 A.II and the measured CM patterns in Fig. 3.5 B I to V. The experimental patterns were obtained from five different guinea pigs. The stimulus parameters in all five cases were essentially the same, thus the stimulus frequency varied only from 13.5 kHz to 14.5 kHz, and the stimulus intensity varied from 80 to 90 db SPL. All experimental amplitude patterns exhibit a distinct minimum around electrodes 6 and 7 and a flat maximum around electrodes 8 and 9. In the region of the amplitude minimum all patterns show the characteristic large phase variation. The overall phase change along the array amounts in the five cases to 285, 320, 276, 350, 240 respectively. For the comparison between the theoretical and experimental CM patterns, we consider the theoretical pattern over a length of 1.65 mm equal to the width of the electrode array. The overall phase variation in this length interval of the theoretical curve has a value of 280. Undoubtedly there is very good quantitative agreement between the theoretical and experimental curves.

In order to show the quantitative fit more clearly the measured CM pattern of Fig. 3.5 B.I was redrawn along with the theoretical pattern of Fig. 3.5 A II in the same diagram in Fig. 3.6. The excellent agreement between the hypothetical and the measured CM patterns, well documented in Fig. 3.6, suggests the following logical conclusion: the hypothetical pattern $H(x)$ from which the hypothetical CM(x_0) in Fig. 3.6 was deduced must approximate very closely to the real hair-cell output pattern which produced the measured CM distribution in Fig. 3.6.

Thus in the following discussion we shall assume that the hair-cell output pattern at stimulus frequencies from 13.5 kHz to 14.5 kHz can be represented by the hypothetical pattern "$H(x)$" given in Fig. 3.5 A I. (Writing $H(x)$ with inverted commas indicates that "$H(x)$" is considered to be an approximation to the real curve.)

shape with increasing frequency and the wave lengths become shorter Thus the excitation pattern is not simply translated towards the basal end. However over a very narrow frequency range we may use the shift in the CM pattern as a first approximation.

We may conclude from the above discussion that G_1 describes adequately the relationship between stimulus frequency and the locus of maximum excitation in the basal part of the guinea pig's cochlea. By expressing x in terms of f we obtain G_1 in the form:

$$G \quad x = 18.5 - \frac{1}{0\ 134} \log\left(\frac{f}{166.2} + 1\right)$$

at high stimulus frequencies

$$\frac{f}{166.2} \gg 1$$

thus

$$x \approx 18.5 - \frac{1}{0\ 134} \log \frac{f}{166.2} \qquad (9\,c)$$

According to equation (9 c) there exists a linear relationship between x and the logarithm of stimulus frequency An octave change in frequency corresponds to a spatial shift Δx of the location of the maximum excitation given by (9 c):

$$\Delta x = \frac{1}{0\ 134} \log 2 = 2.25$$

Thus the maximum shifts by 2.25 mm per octave This is the information needed for the deduction of the frequency response curve from $H(x)$ It enables us to express the slopes of the response curve in db per octave.

Fig. 3 9 shows the envelope of the pattern $H(x)$ corresponding to the experimental CM pattern measured at 13.5 kHz, 90 db. ("$H(x)$ in Fig. 3 9 is the same pattern shown in Fig. 3 4.*B* and Fig. 3 4.*A* I) Let us consider the frequency response curve of the point $M(13.5)$ at which $H(x)$ has its maximum. Increase in stimulus frequency causes a basal shift of the

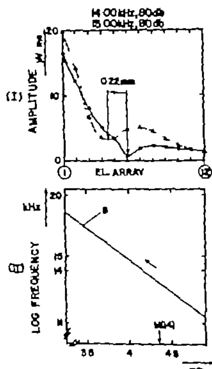


Fig 3.8 The shift in the CM amplitude minimum is taken as measure for the shift in the envelope maximum of the corresponding $H(x)$

I. For an increase in stimulus frequency from 14 to 15 kHz the CM amplitude minimum shifts by approximately 0.22 mm. ●—● CM amplitude pattern at 14 kHz, 80 db ▲—▲ CM amplitude pattern at 15 kHz, 80 db.

II. Stimulus frequency on log. scale versus locus of maximum displacement. G is plotted over the space interval given by the width of the electrode array $M(14)$ denotes the position of the envelope maximum of $H(x)$" at 14 kHz according to K in Fig. 3 7 The spatial shift of 0.22 mm defines the 15 kHz point in the graph. The shift tendency follows G 14 kHz point; ▲ 15 kHz point inferred from the shift in CM amplitude minimum.

pattern $H(x)$" and the output at $M(13.5)$ drops according to the apical slope of $H(x)$ Similarly with decrease in stimulus frequency the output at $M(13.5)$ falls according to the basal slope of $H(x)$" Thus the apical slope of $H(x)$ defines the high frequency branch and the basal slope of $H(x)$" defines the low frequency branch of the frequency response curve.

We can express the slopes of the envelope of $H(x)$" in db per mm. This logarithmic slope

data of von Békésy slightly better than G_1 however it does not take into account the high frequency response of the guinea pig's cochlea Greenwood established G_1 in reference to the finding of Pestalozza & Davis (1956) These investigators obtained electrical responses from the guinea pig s cochlea for stimulus frequencies up to 50 kHz. More recently Wever et al (1963) confirmed 50 kHz as the upper frequency limit of the guinea pig s hearing range by electrical recordings from the cochlea. Thus it appears that G_1 is the more likely function at least as far as the basal part of the cochlea is concerned

There is some more evidence in favour of G_1 Davis and associates (1953) determined histologically the injury of cochlear structures after exposing the guinea pig s ear to intense tones. The injury due to an 8 kHz tone was located in the upper third of the first turn G_1 is compatible with this result Smith & Wever (1949) registered an injury of the organ of Corti centered at 5 mm from the basal end of the membrane after excessive exposure to a 10 kHz tone It can be seen from Fig 3 7 that this result marked SW agrees with G_1 Neubert & Wüstenfeld (1955) explored the extension of the excitation pattern along the basilar membrane by observing the swelling of hair cell nuclei after exposing the guinea pig's ear to prolonged tonal stimuli For a stimulus of 15 kHz and 60 db SPL, lasting for 120 minutes, they reported swelling of nuclei over a well defined region in the basal turn Their result is plotted in Fig. 3 7 marked NW It can be seen that NW overlaps with both functions G_1 and G_2 On the basis of the asymmetric shape of the excitation pattern which has a flat basal and a steep apical slope one would expect that the maximum displacement in the case NW would rather fall on G_1 than on G_2 Thus the evidence mentioned so far fully supports G_1 as the function relating stimulus frequency and locus of maximum displacement in the basal turn

Johnstone & Boyle (1967) and Johnstone (1969) measured with the Mössbauer technique the frequency of maximum response at three points along the basal part of the guinea pig s membrane Their results are shown in Fig 3 1 marked J The results neither fall exactly on G_2 nor on G_1 In the region of the membrane where the results were obtained one would predict from G_1 a frequency of maximum response around 30 kHz. The measured values, however give best frequencies around 20 kHz. It is tempting to speculate that this lowering in the maximum response frequency could be due to the presence of the Mössbauer probe. It is not unlikely that the probe loads the membrane increasing the mass of the system locally Such an increase in the inertia component of the system would inevitably reduce the frequency of maximum response to lower values.

It is interesting to consider the position of the maximum amplitude in "$H(x)$" in relation to G_1 It follows from the fitting procedure outlined in the previous section that the maximum in $H(x)$" occurs between electrodes 8 and 9 (see also Fig 3.9) In Fig 3 7 the location of the maximum is marked M The stimulus frequency (13.5 kHz to 14.5 kHz) and M define our results in Fig 3 7 (marked K) It can be seen that our results agree very well with G_1

There is a further property of our data which agrees with G_1 Fig. 3 8 I shows a shift (0 22 mm) of the minimum in the CM amplitude pattern in the basal direction for an increase in stimulus frequency from 14 to 15 kHz. Let us take this shift in the CM pattern as a direct measure of the shift of the envelope in the corresponding "$H(x)$" In Fig 3 8.II the location of the maximum M(14) for the 14 kHz pattern $H(x)$" is plotted according to Fig 3 7 The 15 kHz point in Fig. 3 8 II was obtained with the above shift of 0.22 mm in the basal direction It is obvious that the tendency of the basal shift follows G very closely Of course the shift in the minimum of the CM pattern is a very rough measure of the shift in the corresponding "$H(x)$" because the excitation pattern along the membrane changes

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at high stimulus frequencies

$$\frac{f}{166.2} > 1$$

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Fig 3.9 shows the envelope of the pattern $H(x)$ corresponding to the experimental CM pattern measured at 13.5 kHz, 90 db. ("$H(x)$" in Fig. 3.9 is the same pattern shown in Fig. 3.4.*B* and Fig 3.4.*A* 1). Let us consider the frequency response curve of the point *M*(13.5) at which $H(x)$ has its maximum. Increase in stimulus frequency causes a basal shift of the pattern $H(x)$ and the output at *M*(13.5) drops according to the apical slope of "$H(x)$". Similarly with decrease in stimulus frequency the output at *M*(13.5) falls according to the basal slope of $H(x)$" Thus the apical slope of $H(x)$ defines the high frequency branch and the basal slope of $H(x)$" defines the low frequency branch of the frequency response curve.

We can express the slopes of the envelope of $H(x)$" in db per mm. This logarithmic slop

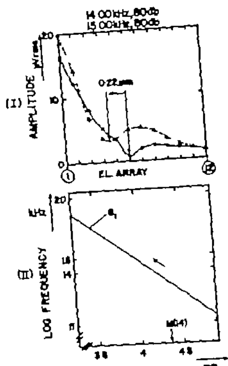


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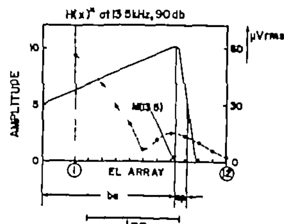


Fig. 3.9. Amplitude envelope of $H(x)$ redrawn from Fig. 3.4 B and Fig. 3.5.A.I. The relative amplitude of $H(x)$ is indicated on the left hand scale. In order to emphasize the deductive source of $H(x)$ we have plotted the experimental CM amplitude pattern obtained at 13.5 kHz, 90 db for comparison (CM pattern replotted from Fig. 3.5 B.I. and Fig. 3.6.). The CM amplitude is given in μV rms on the right hand scale. Thus $H(x)$ is considered a close approximation to the shape of the real hair-cell output distribution which produced the experimental CM pattern. The position of the envelope maximum of $H(x)$ is marked $M(13.5)$. In $H(x)$ the amplitude falls by 6 db over a distance $ba = 1.45$ mm in the basal and a distance $ap = 0.124$ mm in the apical direction. —— envelope of $H(x)$ --- experimental CM amplitude pattern at 13.5 kHz, 90 db.

measure is strictly speaking not absolutely correct because it requires an exponential shape of the envelope. We used a *triangular* shape of envelope in the fitting procedure for convenience. However the logarithmic measure is sufficiently accurate for our purposes. The amplitude in "$H(x)$" drops by 6 db in the basal direction over a distance $ba = 1.45$ mm and in the apical direction over a distance $ap = 0.124$ mm. Thus we obtain as numerical values for the slopes in "$H(x)$"

$$\text{basal slope: } \frac{6}{1.45}\frac{\text{db}}{\text{mm}}$$

$$\text{apical slope: } \frac{6}{0.124}\frac{\text{db}}{\text{mm}}$$

An octave change in stimulus frequency causes a shift of "$H(x)$" by 2.25 mm. It follows for the high and low frequency slope of the response curve of $M(13.5)$

$$\text{low frequency slope: } \frac{6}{1.45} 2.25 \frac{\text{db}}{\text{mm}}\frac{\text{mm}}{\text{octave}} = 9.3\frac{\text{db}}{\text{octave}}$$

$$\text{high frequency slope: } \frac{6}{0.124} 2.25 \frac{\text{db}}{\text{mm}}\frac{\text{mm}}{\text{octave}} = 109\frac{\text{db}}{\text{octave}}$$

The slope values of the frequency response curve derived from "H(x)" are consistent with mechanical data obtained with the Mössbauer technique. This clearly indicates that "$H(x)$" must be a close approximation to the excitation pattern along the membrane.

"$H(x)$" was established by comparing a theoretical CM(x) with experimental CM patterns measured at an SPL of 80 to 90 db (see Fig. 3.5). Thus "$H(x)$" represents the hair-cell output distribution at these stimulus intensities. The question arises whether the shape of "$H(x)$" can be regarded as a close approximation to the shape of the excitation pattern at stimulus intensities less than 80 to 90 db SPL. This question can be answered by considering the shape of the CM pattern at different sound pressures, say from 35 to 85 db SPL. Provided *there is no significant change in the shape* of the CM pattern we can safely assume that the shape of the corresponding "$H(x)$" does not alter significantly with increasing sound pressure (up to 90 db SPL).

The result of such an experiment is shown in Fig. 3.10 A. The CM amplitude pattern of a 13 kHz tone is displayed for SPLs ranging from 35 to 85 db. The amplitudes are plotted on a logarithmic scale and it can be seen that all electrode outputs exhibit perfectly linear intensity functions up to about 65 db. It implies, of course, that the shape of the CM pattern and that of the corresponding "$H(x)$" does not change because all electrode outputs grow in equal proportion. This is not at all surprising since the linear dependence of CM on sound pressure up to 60 or 70 db is a well known fact. More interesting, however, is the

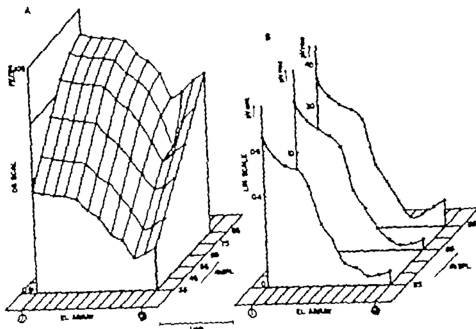


Fig. 3.10. CM amplitude pattern at 13 kHz measured for SPLs from 35 to 85 db

(A) CM amplitude plotted on logarithmic scale. The intensity functions of all electrode outputs are linear up to about 65 db. Thereafter onset of non-linearity and slight differential growth of electrode outputs.

(B) CM amplitude patterns for 35, 65 and 85 db drawn on linear scale. Output at 1 is given equal height in the patterns in order to facilitate comparison between the shape of the patterns. There is only a slight relative increase in the outputs of the apical part of the array at 85 db relative to the lower intensity patterns.

region between 65 and 85 db, the region of onset of nonlinearity. According to Fig. 3.10.A the intensity functions of the different electrodes become slightly different. At 11 and 12 linearity persists, at the remaining electrodes slight deviation from linearity is noticeable. Thus it is clear that the corresponding $H(x)$" underwent slight alteration in shape between 65 and 85 db. Nevertheless, this change in shape must have been very small indeed. In Fig. 3.10.B the 35, 65 and 85 db patterns were redrawn on a linear amplitude scale. In the drawing the output 1 is given equal height in the three patterns in order to facilitate comparison of their shapes. It is obvious that the shapes are almost identical with only a slight growth in the distant electrodes at 85 db relative to the lower intensity patterns.

We can safely assume that the shape of $H(\ )$ is a close approximation to the shape of the hair-cell output pattern for sound pressures up to 90 db SPL. The frequency response curve deduced from $H(x)$ at 90 db is therefore also valid at lower SPLs.

Inversion of the spatial filter operation

The mathematical method used so far was essentially a graphical procedure. We assumed a certain pattern $H(x)$; we calculated the corresponding theoretical $CM(x)$ and compared it with experimental curves. In the event of a good agreement between the theoretical $CM(x)$ and an experimental CM pattern we postulated $H(x)$ as a close approximation to the real hair-cell output pattern along the basilar membrane. This way of operation proved very successful and our data suggest an $H(x)$" fully compatible with the information about the mechanical behaviour of the membrane in the basal turn.

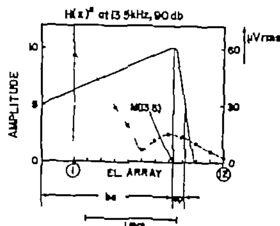


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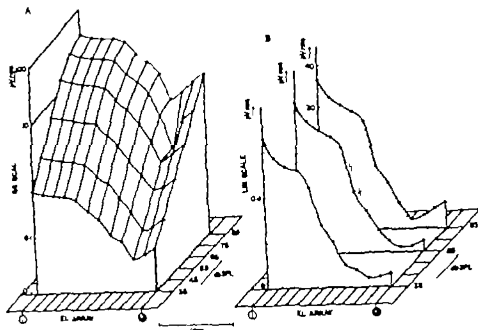


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We can safely assume that the shape of $H(x)''$ is a close approximation to the shape of the hair-cell output pattern for sound pressures up to 90 db SPL. The frequency response curve deduced from $H(x)''$ at 90 db is therefore also valid at lower SPLs.

Inversion of the spatial filter operation

The mathematical method used so far was essentially a graphical procedure. We assumed *a certain pattern* $H(x)'$ we calculated the corresponding theoretical $CM(x)$ and compared it with experimental curves. In the event of a good agreement between the theoretical $CM(x)$ and an experimental CM pattern we postulated $H(x)$ as a close approximation to the real hair-cell output pattern along the basilar membrane. This way of operation proved very successful *and our data suggest an* $H(x)''$ *fully* compatible with the information about the mechanical behaviour of the membrane in the basal turn.

However the graphical method is awkward and time-consuming and therefore not well suited for the analytical treatment of large amounts of data obtainable with the multi electrode technique. This applies especially to the studies of variations in the shape of "$H(x)$" in the nonlinear region of hair-cell operators. It also applies to the investigation of alterations of hair-cell output after overstimulation. We found it therefore highly desirable to develop a way of deducing analytically from any measured CM pattern the corresponding "$H(x)$". Given the function $H(x) = f(\mathrm{CM}(x), W(x))$ explicit in $H(x)$, we were theoretically speaking in a position to derive from any measured $\mathrm{CM}(x)$ the corresponding $H(x)$. In view of this situation my colleague H. F. Ross, suggested matrix inversion as one of the possible ways to derive $H(x)$ directly from the data. In fact it was this original formulation of the deduction problem in matrix form which demonstrated mathematically the possibility for obtaining $H(x)$. Yet, for practical reasons matrix inversion is not the most suitable mathematical technique. It cannot be written in closed form, hence it does not display in explicit fashion the connections involved in the deduction process. From the point of view of the experimenter it is important to know explicitly these connections because they allow the judgement over the practicability of the deduction.

The spatial filter operation can be inverted mathematically and $H(x)$ can be expressed explicitly as a function of $\mathrm{CM}(x)$ and $W(x)$. I obtained the expression for $H(x)$ by means of the following mathematical steps

Given $\quad \mathrm{CM}(x) = H(x) * W(x) \qquad (1)$

and $\quad W(x) = \exp(-\lambda|x|) \qquad (6)$

By taking the Fourier Transform of equation (1)

$$F_{\mathrm{CM}} = F_H \cdot F_W$$

(F denotes the Fourier Transform)

thus $\quad F_H = \dfrac{F_{\mathrm{CM}}}{F_W} \qquad (1a)$

The Fourier transform of equation (6):

$$F_W = \frac{2\lambda}{\lambda^2 + \nu^2} \qquad (6a)$$

where ν is the independent variable in the Fourier domain. It denotes spatial frequency. By inserting (6 a) into (1 a)

$$F_H = F_{\mathrm{CM}}\left[\frac{\lambda^2 + \nu^2}{2\lambda}\right] = \frac{\lambda}{2} F_{\mathrm{CM}} + \frac{\nu^2}{2\lambda} F_{\mathrm{CM}} \qquad (1b)$$

By taking the Inverse Fourier Transform of (1 b) we obtain

$$H(x) = \frac{\lambda}{2}\mathrm{CM}(x) - \frac{1}{2\lambda}\frac{d^2\mathrm{CM}(x)}{dx^2} \qquad (1\,A)$$

(In the case of an asymmetrical weighting function with different attenuation values in the apical and basal directions $H(x)$ takes a slightly different form

with

$$W(x) = \begin{cases} \exp(-\lambda_1|x|) & x < 0 \\ \exp(-\lambda_2|x|) & x > 0 \end{cases}$$

it follows

$$H(x) = \frac{\lambda_1 \lambda_2}{\lambda_1 + \lambda_2}\mathrm{CM}(x) + \frac{\lambda_1 - \lambda_2}{\lambda_1 + \lambda_2}\frac{d\mathrm{CM}(x)}{dx} - \frac{1}{\lambda_1 + \lambda_2}\frac{d^2\mathrm{CM}(x)}{dx^2}$$

This expression was obtained in the same way as (1 A). In the case $\lambda_1 = \lambda_2 = \lambda$ equation (1 A) results. Since von Békésy reported symmetrical attenuation we only considered (1 A).)

In equation (1 A) $H(x)$ appears as the linear combination of $\mathrm{CM}(x)$ and $\mathrm{CM}(x)''$. For a large λ, i.e. strong electrical attenuation of signal along the cochlea, we can approximate equation (1 A) by the expression in (1 B)

$$H(x) \approx \frac{\lambda}{2}\mathrm{CM}(x), \quad \lambda > 1 \qquad (1\,B)$$

This is an expression valid in the absence of spatial filtering. Due to strong longitudinal attenuation there is no interference of hair cell outputs along the cochlear ducts. In such a situation an electrode output at x directly reflects the corresponding hair-cell activity at x. Equation (1 *B*) is also an approximation in the case of a small second spatial derivative of $CM(x)$:

$$H(x) \approx \frac{\lambda}{2} CM(x), \quad CM(x)'' < 1 \qquad (1\,B)$$

This expression of $H(x)$ applies to some extent to the basalmost part of the excitation pattern along the membrane at low stimulus frequencies. The wavelength is relatively long in this part and negative interference, phase cancellation of hair-cell outputs will be negligible. In the case of small longitudinal attenuation we may approximate equation (1 *A*) by (1 *C*):

$$H(x) \approx -\frac{1}{\lambda} CM(x)'' \quad \lambda < 1 \qquad (1\,C)$$

In this case it is the second spatial derivative of $CM(x)$ which resembles $H(x)$ directly and $CM(x)$ is not itself an index of $H(x)$. Clearly such a situation requires the recording of the second spatial derivative of $CM(x)$ as a measure of $H(x)$. Widely spaced electrodes are completely inadequate because they do not provide any information about the local curvature of the CM pattern—information needed for the calculation of the second derivative. Only by means of closely spaced electrodes do we obtain sufficient numerical information about the shape of the CM pattern to allow the derivation of $CM(x)''$.

In reality λ is neither very much smaller nor very much greater than 1: it has a value of $\lambda = 0.69$ corresponding to a signal attenuation of 6 db per mm. For the deduction of $H(x)$ from experimental CM curves we have to use equation (1 *A*).

$$H(\ ) = 0.35\,CM(\ ) - 0.72\,CM(x) \qquad (1\,A)$$

Mathematically speaking, equation (1 *A*) poses no great problems; it is a neat and simple expression for the relationship between $H(x)$ and $CM(x)$. It will, however, turn out that the requirements on experimental data, implicit in (1 *A*), are too demanding for the practical application of the inverse spatial filter operation. This is due to the second derivative term in (1 *A*) which magnifies any slight irregularity in the measured CM pattern. We can demonstrate the strong influence of $CM(x)''$ on $H(x)$ in a simple hypothetical case. In Fig. 3.11 we considered the shape of $H(x)$ resulting from a hypothetical $CM(x)$ given as a straight line

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$$\text{Given} \quad \mathrm{CM}(x) = H(x) * W(x) \tag{1}$$

$$\text{and} \quad W(x) = \exp(-\lambda|x|) \tag{6}$$

By taking the Fourier Transform of equation (1)

$$F_{CM} = F_H\ F_W$$

(F denotes the Fourier Transform)

thus

$$F_H = \frac{F_{CM}}{F_W} \tag{1 a}$$

The Fourier transform of equation (6):

$$F_W = \frac{2\lambda}{\lambda^2 + \nu^2} \tag{6 a}$$

where ν is the independent variable in the Fourier domain it denotes spatial frequency By inserting (6 a) into (1 a)

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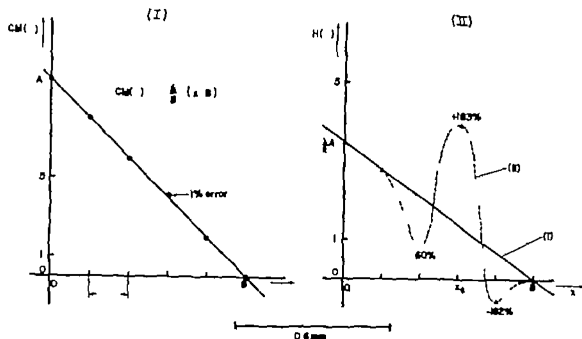


Fig 3 11 Application of the inverse spatial filter operation to a hypothetical CM(*x*).
I. CM(*x*) assumed as

$$CM(x) = -\frac{A}{B}(x - B) \qquad (10a)$$

II The corresponding $H(x)$ has the form

$$H(x) = -\frac{\lambda}{2}\frac{A}{B}(x - B) \qquad (10b)$$

$H(x)$ is drawn in II for $\lambda = 0\ 69$ and it is marked *I* (——) Suppose CM(*x*) was measured with a hypothetical set of electrodes with an interelectrode distanc $\Delta x = 0\ 15$ mm equal to the distance between electrode in the real electrode array Suppose further that elec trode at x_i records an 'error potential' which deviate from the straight line by 1% This 1% error produce large deflections in the deduced $H(x)$ (II, – –) around the ideal straight line. The deviation by 1% at x in $CM(x)$ causes large deviations in $H(x)$: at x_{-1} it is -60% at x $+183\%$ and at x_{+1} it is -182% Thus small ripples in any given CM(*x*) produce large deflec tions of short wavelength in the deduced $H(x)$

This result is somewhat disturbing, apart from the fact that it demonstrates the im practicability of the direct deduction of $H(x)$ from CM(x) We may explain the necessity of this result in a simple model based upon the analogy between spatial filtering and linear electrical filters. In Fig 3 12 $H_i(x)$ and CM (x) represent the input and output functions of the

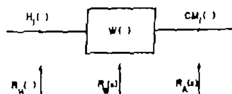


Fig 3.12 Model for the inverse spatial filtering operation. In the ideal situation the input function H (x) can be reconstructed from the output function $CM_i(x)$ In reality 'ripple noise' is present.
$R_A(x)$: ripple noise in the recording system.
R (x): ripple noise due to inadequate $W(x)$.
$R_H(x)$: ripple noise present in the input function $H(x)$.

spatial filter in the ideal situation with no disturbing effects present.

$W(x)$ denotes the spatial impulse response of the spatial filter Since the Fourier Transform of $W(x)$ equals the spatial frequency response of the spatial filter we can write according to equation (6 *a*)

$$F_W = \frac{2\lambda}{\lambda^2 + \nu^2} \qquad (6a)$$

It follows from (6 *a*) that high spatial fre quencies ν are much more attenuated than low ones or when expressed in terms of wave length that short wavelengths in $H(x)$ are much more attenuated than long wavelengths. Thus the low spatial frequency components of H (x) will be dominating the appearance of the pattern $CM_i(x)$ and high spatial frequency components will just lead to slight 'ripple like' undulations. Nevertheless the input $H_i(x)$ can

be recovered unequivocally from $CM_i(x)$ by virtue of the second spatial derivative of $CM_i(x)$ which enhances the ripples of short wavelength, i.e. of high spatial frequency.

However in the real situation the reconstruction of the input $H_i(x)$ from experimental data runs into difficulties because we encounter small irregularities in the output pattern. Let us call these irregularities spatial 'ripple noise' $R(x)$. Thus the experimental CM pattern can be represented as the sum of the ideal pattern $CM_i(x)$ and $R(x)$.

$$CM(x) = CM_i(x) + R(x)$$

When $R(x)$ has wavelengths in the order of magnitude of the short wavelengths in the input pattern it is clearly impossible to obtain an unambiguous reconstruction of the input pattern from the output data. Thus if we want to deduce from experimental CM patterns the corresponding hair-cell output pattern we face the problem of having to identify $R(x)$ or in other words, we must distinguish between ripple noise $R(x)$ and the ideal pattern $CM_i(x)$. This cannot be an easy task, because all we have at hand is $CM(x)$.

The identification of small ripples in experimental CM patterns as ripple noise $R(x)$ would be quite simple provided the ripples occurred at random. Thus if slight undulations in CM patterns are not reproducible within one experimental session and in different experiments we have a certain right to disregard ripples in any one particular CM pattern. By fitting the CM patterns with smooth curves, omitting irregularities of short wavelength we could approximate to $CM_i(x)$ and proceed to calculate $H_i(x)$.

However the ripple patterns shown by our recordings exhibit a high degree of stability and reproducibility. They were found consistently in all preparations. The ripples occurred usually in the regions centered around electrodes 2, 5, 9. This gives an average ripple length of approximately 0.5 mm. It is interesting to note that the ripple length did not change significantly with stimulus frequency. By using

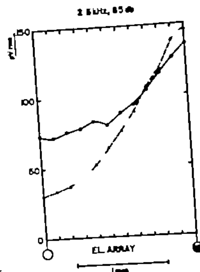


Fig. 3.13 CM amplitude distribution at 2.5 kHz, 85 db measured before and after overstimulation with 13 kHz, 130 db for 3 min. While the pre-traumatic curve contains small ripples the post-traumatic curve is very smooth. This indicates that the ripples are not dependent on the recording system, i.e. they are not introduced by nonuniformity in electrode channels. $R_s(x)$ can be excluded as source for ripple noise. ●—● 2.5 kHz CM amplitude pattern before overstimulation with 13 kHz tone; ▲—▲ 2.5 kHz CM amplitude pattern after overstimulation with 13 kHz tone.

equation (1 A) we calculated patterns $H(x)$ which had more than one maximum in the envelope: the ripple pattern seen in the experimental CM patterns appeared strongly enhanced in the deduced $H(x)$.

For the discussion of the question whether the ripples in the experimental CM pattern represent a real phenomenon or just an artefact it is convenient to distinguish three group of possible sources for the ripples (see Fig 3.12). $R_H(x)$ denotes the ripple noise occurrin in the input signal $H(x)$ itself. $R_W(x)$ designate ripple noise due to the inadequacy of the spatial filter function $W(x)$. $R_s(x)$ designate ripple noise due to imperfection in the recording system, as for instance slight nonuniformity of different electrode channels.

$R_s(x)$: We can rule out $R_s(x)$ on the basis o the following observation. Fig. 3.13 shows th

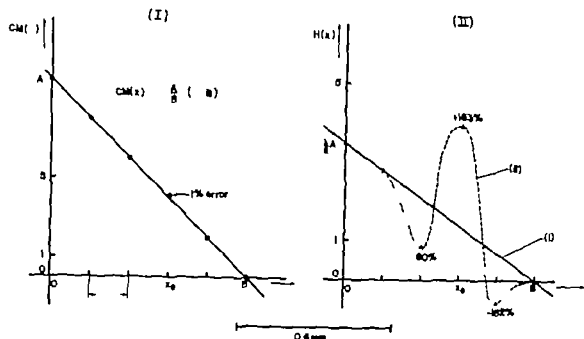


Fig 3 11 Application of the inverse spatial filter operation to a hypothetical CM(x)
I. CM(x) assumed as.

$$\mathrm{CM}(x) = -\frac{A}{B}(x-B) \qquad (10\,a)$$

II The corresponding $H(x)$ has the form.

$$H(x) = -\frac{\lambda}{2}\frac{A}{B}(x-B) \qquad (10\,b)$$

$H(x)$ is drawn in II for $\lambda = 0.69$ and it is marked *I* (——) Suppose CM(x) was measured with a hypothetical set of electrodes with an interelectrode distance $\Delta x = 0.15$ mm equal to the distance between electrodes in the real electrode array Suppose further that electrode at x records an error potential' which deviates from the straight line by 1% This 1% error produces large deflections in the deduced $H(x)$ (II, --) around the ideal straight line. The deviation by 1% at x_0 in CM(x) causes large deviations in $H(x)$: at x_{-} it is −60% at x +183% and at x it is −182% Thus small ripples in any given CM(x) produce large deflections of short wavelength in the deduced $H(x)$.

This result is somewhat disturbing apart from the fact that it demonstrates the impracticability of the direct deduction of $H(x)$ from CM(x) We may explain the necessity of this result in a simple model based upon the analogy between spatial filtering and linear electrical filters. In Fig 3 12 $H(x)$ and $CM_i(x)$ represent the input and output functions of the spatial filter in the ideal situation with no disturbing effects present

$W(x)$ denotes the spatial impulse response of the spatial filter Since the Fourier Transform of $W(x)$ equals the spatial frequency response of the spatial filter we can write according to equation (6 a)

$$F_W = \frac{2\lambda}{\lambda^2 + \nu^2} \qquad (6\,a)$$

It follows from (6 a) that high spatial frequencies ν are much more attenuated than low ones, or when expressed in terms of wave length that short wavelengths in $H(x)$ are much more attenuated than long wavelengths. Thus the low spatial frequency components of $H(x)$ will be dominating the appearance of the pattern $CM_i(x)$ and high spatial frequency components will just lead to slight ripple like undulations. Nevertheless the input $H(x)$ can

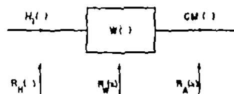


Fig 3 12 Model for the inverse spatial filtering operation. In the ideal situation the input function $H(x)$ can be reconstructed from the output function $CM_i(x)$. In reality 'ripple noise' is present.
$R_A(x)$: ripple noise in the recording system
$R_W(x)$: ripple noise due to inadequate $W(x)$.
$R_H(x)$: ripple noise present in the input function $H(x)$

be recovered unequivocally from $CM_i(x)$ by virtue of the second spatial derivative of $CM_i(x)$ which enhances the ripples of short wavelength, i.e. of high spatial frequency.

However in the real situation the reconstruction of the input $H_i(x)$ from experimental data runs into difficulties because we encounter small irregularities in the output pattern. Let us call these irregularities spatial 'ripple noise' $R(x)$. Thus the experimental CM pattern can be represented as the sum of the ideal pattern $CM_i(x)$ and $R(x)$.

$$CM(x) = CM_i(x) + R(x)$$

When $R(x)$ has wavelengths in the order of magnitude of the short wavelengths in the input pattern it is clearly impossible to obtain an unambiguous reconstruction of the input pattern from the output data. Thus if we want to deduce from experimental CM patterns the corresponding hair-cell output pattern we face the problem of having to identify $R(x)$, or in other words, we must distinguish between ripple noise $R(x)$ and the ideal pattern $CM_i(x)$. This cannot be an easy task, because all we have at hand is $CM(x)$.

The identification of small ripples in experimental CM patterns as ripple noise $R(x)$ would be quite simple provided the ripples occurred at random. Thus if slight undulations in CM patterns are not reproducible within one experimental session and in different experiments we have a certain right to disregard ripples in any one particular CM pattern. By fitting the CM patterns with smooth curves, omitting irregularities of short wavelength we could approximate to $CM_i(x)$ and proceed to calculate $H_i(x)$.

However the ripple patterns shown by our recordings exhibit a high degree of stability and reproducibility. They were found consistently in all preparations. The ripples occurred usually in the regions centered around electrodes 2, 5, 9. This gives an average ripple length of approximately 0.5 mm. It is interesting to note that the ripple length did not change significantly with stimulus frequency. By using

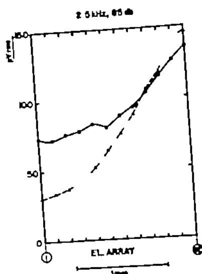


Fig. 3.13 CM amplitude distribution at 2.5 kHz, 85 db, measured before and after overstimulation with 13 kHz, 130 db for 3 min. While the pre-traumatic curve contains small ripples the post-traumatic curve is very smooth. This indicates that the ripples are not dependent on the recording system, i.e. they are not introduced by nonuniformity in electrode channels. $R_S(x)$ can be excluded as a source for ripple noise. ●—● 2.5 kHz CM amplitude pattern before overstimulation with 13 kHz tone, ▲—▲ 2.5 kHz CM amplitude pattern after overstimulation with 13 kHz tone.

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CM amplitude pattern in response to a stimulus of 2.5 kHz and 85 db SPL. The CM pattern was measured before and after exposing the animal for 3 min to a tone of 13 kHz, and 130 db. The overstimulation with 13 kHz caused impairment of hair-cell activity and consequently there is a marked difference between the two patterns acquired before and after overexposure. As far as the smoothness of the patterns is concerned we can see small irregularities in the pattern measured before overstimulation. In the CM pattern obtained after overstimulation, however, ripples are practically absent. (It may be mentioned here that we always observed relatively smoother CM patterns after subjecting of the guinea pig's ear to excessive stimulation.) The perfectly smooth CM pattern after overstimulation in Fig. 3.13 demonstrates that the electrode array does record patterns without introducing irregularities; thus it is clear that we must seek other reasons for the ripple structure.

$R_B(x)$. A potential source for the ripples in the CM patterns is the curved course of the basilar membrane and the cochlear canals. It is not unlikely that the bending of the cochlear structures has an effect on the potential field in the perilymph. This implies that a straight-lined out core conductor model for the current spread in the basal turn is only valid as a first approximation and that the weighting function $W(x) = \exp(-\lambda |x|)$ is not completely adequate for the description of electrical signal attenuation. If it is true that $W(x)$ does not reflect exactly the spatial properties of the potential spread it is clear that the application of $W(x)$ in the inverse spatial filter operation introduces spatial ripple noise. The original distribution of hair-cell activity can only be correctly reconstructed from CM data provided we know exactly the conditions of potential spread along and across the canals. Since we do not know these conditions exactly—not with the accuracy required for the mathematical deduction of $H(x)$—we cannot estimate the influence of $R_B(x)$ on the resulting $H(x)$, i.e. we cannot estimate the error introduced by the use of a weighting function $W(x)$ which is only a first approximation to the signal spread along the canals.

$R_H(x)$. The ripples observed in the experimental CM patterns could be due to undulations of short wavelength in the hair-cell output pattern; thus they could represent the spatially filtered image of an amplitude pattern modulated on top of the excitation pattern along the membrane. We cannot exclude the possibility that such a modulation pattern could arise from the disturbance of the system by the multi-electrode technique although as was pointed out in Section 2, any significant disturbance of the system seems unlikely. The alternative possibility, namely that the modulation pattern is a genuine property of the system, seems rather odd. However there is some evidence from another source which seems to indicate a certain spatial periodicity structure in the envelope of the excitation pattern. Engström et al. (1966) assessed hair-cell damage along the basilar membrane after exposing animals repeatedly to high intensity noise. Their findings fully agree with the results of other investigators, thus the frequency spectrum of the noise determines the location of maximum damage, the extent and degree of damage depending on SPL. In addition, however, they observed a second feature in the damage pattern:

> The damage often has a kind of maximum center which is bordered by a region of undamaged cells. Beyond this is seen a zone of more pronounced damage on both sides of the center and then a zone of scattered damage. In some cases we have had the impression that the damage had a kind of periodical distribution around a center.

In view of this observation the ripple structure seen in the CM patterns assumes a certain relevance. It is unfortunate that Engström et al. did not mention the wavelength of the spatial periodicity in the damage distribution allowing the comparison with the ripple length of approximately 0.5 mm.

Despite extensive efforts I was not successful in establishing unequivocal criteria for the separation of the ripple noise $R(x)$ from the ideal $CM_i(x)$ in the experimental curves. Without a clear insight into the mechanisms causing the ripples we cannot identify accurately in any one particular CM pattern those features which are due to $R(x)$ and those which represent genuine information about short wavelengths in the hair-cell output pattern. Analytic treatment of the compound experimental CM pattern, with the equation for the inverse spatial filtering, however unduly magnifies the spatial frequency components due to $R(x)$. This clearly indicates the serious limitations of a purely analytical approach in the evaluation of CM data.

The previously outlined, graphical method produced a close approximation $H(x)$ to the hair-cell output pattern along the basilar membrane $H(x)$ was in accordance with data about the mechanical frequency response curves. In the approximative sense the CM patterns fully support $H(x)^*$ yet, in the strict mathematical sense we cannot prove that $H(x)^*$ is the only possible function which corresponds to the measured CM patterns. The question of uniqueness remains unsolved. The inversion of the spatial filter operation gave a neat expression but was not very useful in the treatment of data. By analogy to linear filter theory we can appreciate the difficulties: it cannot be an easy task to reconstruct the input signal from the output signal of a low pass filter in the presence of noise.

C. NONLINEAR STUDIES

The multi-electrode technique is well suited for the study of local changes in the hair-cell output pattern $H(x)$. Such changes influence the whole CM pattern, the influence being strongest in the region of the occurring change and progressively less on either side. We can conveniently express this spatially weighted influence of local changes in $H(x)$ by means of equation (1). Let $CM_B(x)$ be the distribution corresponding to $H_B(x)$:

$$CM_B(x) = \int_{-\infty}^{\infty} H_B(z)\, W(x-z)\, dz$$

By a local change, $\Delta H_{BA}(x)$, in the original hair-cell output distribution $H_B(x)$ we obtain the modified $H\ (x)$

where $\Delta H_{BA}(x) = H_B(x) - H_A(x)$

$H\ (x)$ has a corresponding CM distribution $CM\ (x)$.

$$CM_A(x) = \int_{-\infty}^{\infty} H_A(z)\, W(x-z)\, dz$$

Due to the linearity of the spatial filter operation we can write for the difference between $CM_B(x)$ and $CM_A(x)$:

$$CM_B(x) - CM_A(x) = \Delta CM_{BA}(x)$$

$$\begin{aligned}\Delta CM_{BA}(x) &= \int_{-\infty}^{\infty} H_B(z)\, W(x-z)\, dz - \int_{-\infty}^{\infty} H\ (z)\, W(x-z)\, dz \\ &= \int_{-\infty}^{\infty} (H_B(z) - H_A(z))\, W(x-z)\, dz \\ &= \int_{-\infty}^{\infty} \Delta H_{BA}(z)\, W(x-z)\, dz \\ &= \Delta H_{BA}(x) * W(x) \qquad (1\,D)\end{aligned}$$

Thus the change in the CM pattern $\Delta CM_{BA}(x)$ is the spatially filtered image of $\Delta H_{BA}(x)$. It is essential to realize this property of the spatial filtering process: a local modification of a given hair-cell activity distribution does not produce an equally well localized alteration in the corresponding CM distribution. The effect of a local change in $H(x)$ is distributed along the whole CM pattern according to the attenuation function $W(x)$. Clearly this has an important bearing on the experimental study of localized alterations in the hair-cell output

CM amplitude pattern in response to a stimulus of 2.5 kHz and 85 db SPL. The CM pattern was measured before and after exposing the animal for 3 min to a tone of 13 kHz, and 130 db. The overstimulation with 13 kHz caused impairment of hair-cell activity and consequently there is a marked difference between the two patterns acquired before and after overexposure. As far as the smoothness of the patterns is concerned we can see small irregularities in the pattern measured before overstimulation; in the CM pattern obtained after overstimulation, however, ripples are practically absent. (It may be mentioned here that we always observed relatively smoother CM patterns after subjecting of the guinea pig's ear to excessive stimulation.) The perfectly smooth CM pattern after overstimulation in Fig. 3.13 demonstrates that the electrode array does record patterns without introducing irregularities; thus it is clear that we must seek other reasons for the ripple structure.

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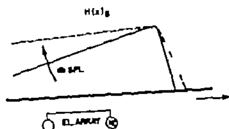


Fig 3.14 Schematic representation of recording situation at 5 kHz. The maximum excitation happens apical to the electrode array The differential growth of $H(x)_s$ in the nonlinear part of the dynamic range is indicated by flattening the basal and apical slopes. Peak-limiting occurs first in the central region of the excitation pattern. Electrode 1 is expected to record relatively more increase in CM amplitude than 12 with increase in SPL.

CM recorded with an electrode situated in the region of maximum displacement. The output of such an electrode is dominated by the contributions from hair-cell generators in this region. Since it is to be expected that saturation of hair-cell outputs with increasing stimulus intensity begins first in the region of maximum displacement it follows that the rate of increase declines much more in this region than in the regions on either side. Out-of phase contributions from the basal and apical parts of the hair-cell output pattern will therefore increase relative to the contributions from the saturated hair-cell generators and a drop in the observed electrode output will result. This rather attractive explanation implies—although not explicitly stated by Whitfield & Ross—that electrodes apical and basal to the considered electrode must continue to record increasing CM because of the continued growth of hair-cell outputs in the apical and basal part of the displacement patterns with increase in sound pressure Our experimental data fully support the hypothesis.

Let us consider the case of $H(x)_f$ that is the hair-cell output distribution at stimulus frequency f In the linear part of the dynamic range $H(x)_f$ increases uniformly with increasing sound pressure and so does the resulting CM(x) (see Fig. 3.10) All hair-cell outputs grow in equal proportion and the shape of $H(x)_f$ remains unaffected. In the nonlinear range characteristic variation of the shape of $H(x)$ must occur due to the growth differential of hair-cell outputs along the basilar membrane In the region of maximum displacement nonlinear limiting of $H(x)_f$ will appear foremost, yet the remaining apical and basal parts of $H(x)_f$ will grow further at unreduced rate with increase in sound pressure. Thus the basal and apical parts of $H(x)$ increase relative to the central part. Such a differential growth of $H(x)_f$ must produce characteristic alterations in the shape of the corresponding CM amplitude and phase pattern The basal and apical parts of $H(x)_f$ will exert increasingly more influence on the CM pattern with increasing SPL. We may expect the following features of CM(x) in the nonlinear part of the dynamic range.

1) The CM in the basal and apical regions of $H(x)_f$ saturates at relatively higher SPLs than the CM in the central region of maximum displacement.

2) The CM in the central region decreases while the CM in the basal and apical regions continues to increase.

3) The tendency of the shape variation of the CM phase pattern indicates the increasing influence of the unsaturated parts of $H(x)_f$ on the CM distribution.

The observable variation of the shape of a CM pattern depends in any one particular case on the position of the electrode array relative to the maximum displacement of the basilar membrane. Fig. 3.14 shows schematically the recording situation at a stimulus frequency of 5 kHz.

The maximum displacement at 5 kHz occurs apical to the electrode array and consequently we observe a CM pattern predominantly influenced by the basal part of $H(x)_5$. We can schematically represent the differential growth of $H(x)_5$ in the nonlinear part of the dynamic range by flattening the basal and apical slopes. Thus with increasing sound pressure electrode 1 will record relatively more increase in CM amplitude than electrode 12. In Fig. 3.15 the

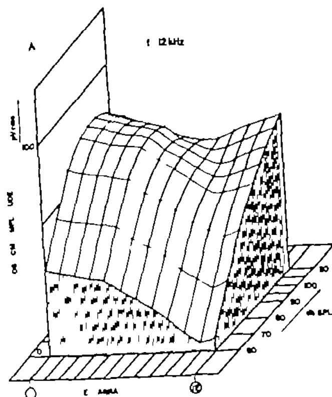
of a given $H(x)$. The information about a local change $\Delta H_{BA}(x)$ is contained in the variation of the *shape* of the *whole* CM pattern and thus concerns both the CM amplitude and the CM phase pattern. Hence for the description of $\Delta H_{BA}(x)$ we require the knowledge of the shape variation of CM(x). Needless to say that the traditional techniques with only a few widely spaced recording points along the cochlea cannot meet this requirement since they do not provide sufficient information about the shape of the CM amplitude and phase pattern in the first place and, of course, even less so about the shape variation of the CM pattern. Although the multi-electrode technique allows the study of CM potentials only over a short length of membrane, nevertheless it provides all information needed for the description of events in this region. The usefulness of the multi-electrode technique in the investigation of local, that is differential changes in hair-cell output is exemplified in the following sections. We have chosen for this study: 1) The differential growth of $H(x)$ in the nonlinear part of the CM intensity function, 2) two-tone interaction, and 3) tonal overstimulation.

In order to facilitate the discussion of the following graphs it is helpful to mention explicitly the dual aspects which will be considered. On the one hand the variations in the shape of CM amplitude and phase patterns indicate the underlying changes in the corresponding hair-cell output pattern. On the other hand the local changes in $H(x)$ which have presumably taken place—in any one specific case—cause a characteristic variation in the shape of the resulting CM pattern; this variation conforms to the concept of spatial filtering.

The CM intensity function

The intensity function, that is the relation between CM amplitude and sound pressure has been studied by many investigators (see for instance Wever 1949, Tasaki et al. 1952, Honrubia & Ward, 1968). It is linear up to about 60 to 70 db SPL; it deviates slightly from linearity between 70 and 90 db SPL and becomes strongly nonlinear beyond 100 db SPL. The saturation shown by the intensity function indicates peak limiting in the potential excursions of hair-cell outputs due to some nonlinear property of the mechano-electric transduction process. Models for the nonlinear behaviour of the CM at high stimulus intensities were suggested by Johnstone & Johnstone (1966) and Engebretson & Eldredge (1968); these models, however, do not provide any explanation for the decrease in the CM at very high intensities. The decline of the CM amplitude at high input levels is a curious feature of the intensity function and it cannot be described by a simple polynomial nonlinearity in the transducer system. Wever (1949) postulated that the drop in the intensity function is the result of the combined effect of two separate mechanisms without, however, clearly specifying these mechanisms. Davis & Eldredge (1959) advanced the hypothesis that longitudinal slippage between the tectorial membrane and the organ of Corti bends—at large sound pressures—the stereocilia of the outer hair cells sideways at right angles to their effective direction of movement. Such a bending of stereocilia, it is argued, could reduce their deflection in the most effective direction and thus cause a drop in hair-cell output. As far as the experimental investigation of such an intricate mechanism is concerned Davis & Eldredge granted that "it is difficult to test or even assess this situation".

Whitfield & Ross (1965) suggested an experimentally testable hypothesis. They proposed phase cancellation between outputs of hair-cell generators as the mechanism responsible for the decrease in CM amplitude at high input levels. In their model the hair-cell output itself is assumed to follow an intensity function with saturation but without decrease at high stimulus intensities. By considering the differences in the rate of increase between hair-cell outputs along the excitation pattern with increase in stimulus intensity they obtained an elegant explanation for the drop in



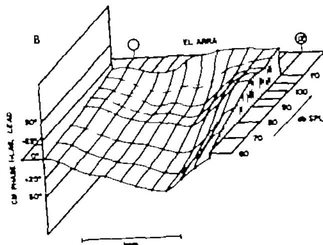


Fig. 3.16 Experimental CM amplitude (A) and phase (B) pattern at 12 kHz as function of SPL. (Data in Figs. 3.16 and 3.15 were obtained in the same guinea pig.)

(A) Note saturation in the basal part of the array: apical CM outputs continue to increase.

(B) At high SPLs the phase values in the apical part of the array dominate the CM phase patterns. This indicates the increasing influence of contributions from the apical part of the hair-cell output distribution on the CM pattern. The contour of 'zero-phase' is drawn into the graph (–). The phase readings are referred to the phase value of 1 at 60 db.

we expect to find an increasing influence of the apical portion of $H(x)$ on the CM pattern recorded with the electrode array. The phase diagram in Fig. 3.16 *B* clearly demonstrates this increasing influence. We observe in this graph a distinct tendency of phase values to shift towards the phases in the apical part of the array: we may say that the apical phase values tend to dominate the shape of the phase pattern with increasing sound pressure.

Two more examples obtained from another guinea pig are shown in Figs. 3.17 and 3.18.

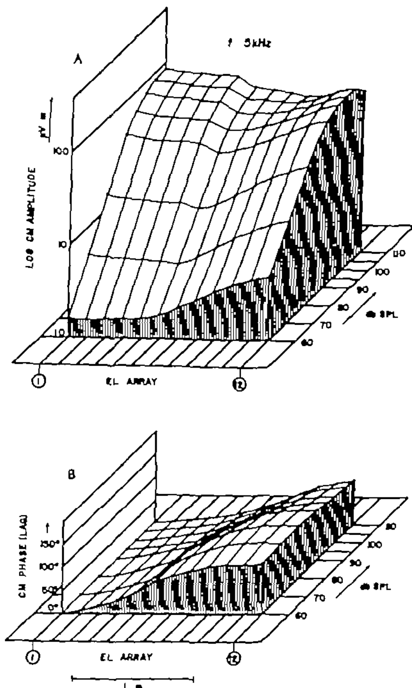


Fig 3.15 Experimental CM amplitude (A) and phase (B) pattern at 5 kHz as a function of SPL.

(A) Note the saturation in the apical part of the array i.e. the part closest to the *maximum displacement at 5 kHz. At* 12 CM amplitude declines while basal electrode outputs continue to increase.

(B) The phase reading are referred to the phase value of 1 at 60 db

experimental CM amplitude and phase patterns at 5 kHz and SPLs from 60 to 110 db are shown It can be seen at once from the 3-dimensional graph that saturation occurs in the apical part of the array that is in the part nearest to the region of maximum displacement It is also interesting to note that the CM amplitude drops at electrode 12 while *for instance at electrode 1 no such drop occurs. Quite the reverse is true in the 12 kHz record shown in Fig. 3 16* (The data in Fig. 3 15 and Fig 3 16 stem from the same animal) Here, electrode 1 records a decrease in CM amplitude, while electrode 12 records continued increase in CM amplitude This is not a surprising situation when we consider the position of the maximum displacement of the basilar membrane at 12 kHz According to Greenwood's formula (*G*) the maximum is located in the region around electrodes 9 and 10 i e within the width of the electrode array Thus in the non-linear part of the dynamic range

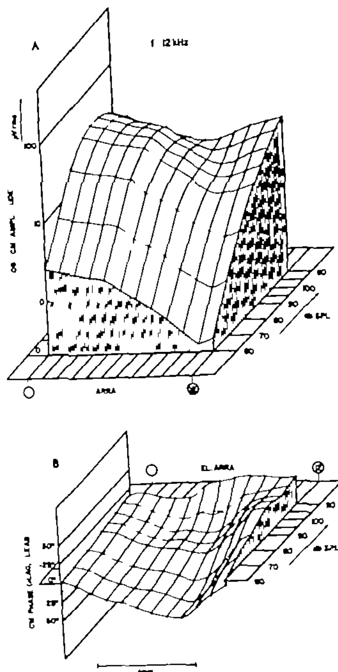


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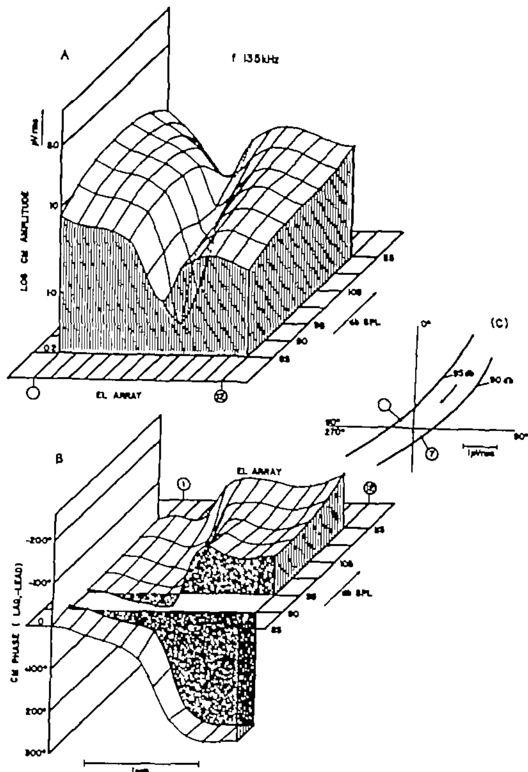


Fig. 3.17. CM amplitude (A) and phase (B) pattern measured at 13.5 kHz as a function of SPL.

(A) Note drop in amplitude in the basal region of the array; the amplitude in the apical region shows continued increase in amplitude.

(B) Notice the growing dominance of apical phase values over phase pattern with increasing SPL. The phase values are referred to that of 1 at 85 db.

(C) Vector representation. Phase reversal occurs between 90 db and 95 db. A short section of the trajectory in the 90 db and 95 db case is shown. The arrow along the two trajectories denotes the apical direction along electrode array. The 90 db trajectory encircles the origin of the vector plane while the 95 db trajectory does not. Output 7 comes very close to the origin. By addition of a small vector with large phase lag to the 90 db trajectory one obtains a trajectory similar to that at 95 db, i.e. with no encirclement of the origin. The additional small vector is thought to represent the relati e increase in the large phase-lag contributions from the apical region of the 13.5 kHz hair-cell output distribution. (Consult Fig. 3.2.D for the ector addition.)

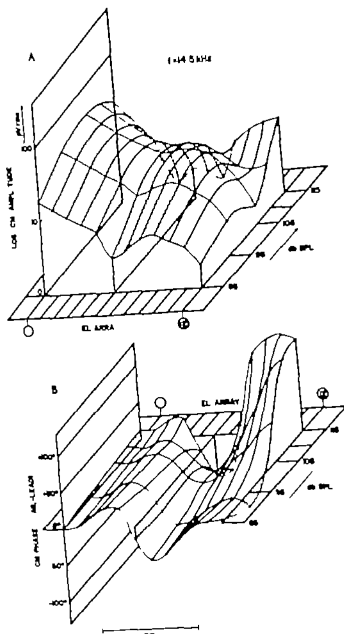


Fig. 3.18 CM amplitude (A) and phase (B) pattern measured at 14.5 kHz as function of SPL. (Figs. 3.17 and 3.18 refer to the same guinea pig.)

(A) Marked increase in CM amplitude in the apical part of the array—the remaining part show marked decrease in amplitude.

(B) Apical phase values clearly dominate CM phase pattern with increase in SPL. The phase values are referred to that of 1 at 85 db.

the stimulus frequency in the two cases was 13.5 kHz and 14.5 kHz respectively. At these frequencies maximum displacement of the basilar membrane occurs in the region around electrodes 8 and 9 and consequently the apical part of $H(x)$ will be dominating the shape variation of the CM distribution along the array with increasing SPL. The 13.5 kHz record in Fig. 3.17 exhibits the characteristic features of this dominating influence in the amplitude as well as the phase patterns. While the basal part of the array shows a drop in CM amplitude the apical part does not. The typical tendency of CM phase values to shift with increasing SPL towards the phase values of the apical part of the array is well shown in the

13.5 kHz phase record. A particularly interesting feature of the 13.5 kHz record is the occurrence of phase reversal between the 90 db and 95 db pattern. At these intensities there is a distinct amplitude minimum at electrode 7. By plotting the data in vector form one obtains trajectories which run very close to the origin; this is indicated in Fig. 3.17 C, where a short section of the trajectory has been drawn for the 90 db and the 95 db pattern. It can be seen that the output of electrode 7 comes very near to the origin of the vector plane. The 90 db trajectory encircles the origin, hence there is progressive phase lag along the electrode array. The 95 db trajectory, however, shows no encirclement and phase reversal within the CM phase pattern must occur. It is apparent that CM patterns with a distinct amplitude minimum are potentially useful in detecting small variations in the shape of the corresponding hair-cell output pattern. A CM amplitude minimum in a case like the one in Fig. 3.17 is the result of mutual, almost total cancellation of contributions from out-of-phase hair-cell generators; in other words at such an amplitude minimum the out-of-phase contributions balance each other. Any slight change in the shape of the hair-cell output distribution will upset the balance and alter the CM pattern in favour of those contributions which showed a relative increase. Thus in the above 13.5 kHz case we would expect a relative increase in the apical part of $H(x)$ with increase in SPL, enhancing the influence of large phase lag contributions on the shape of the CM pattern. We may represent this relative increase by adding a small vector with a large phase lag to all electrode outputs in the 90 db case in which the trajectory encircles the origin. The length of the additional vector diminishes as we proceed towards the basal end of the electrode array. It is obvious that the resulting trajectory will not encircle the origin; hence the resulting 95 db phase pattern will show phase reversal. (The reader may consult Fig. 3.2.D which shows schematically the effect of the above described vector addition.)

The 14.5 kHz record in Fig. 3.18 exhibits once more the characteristic features mentioned in the discussion of the previous diagrams. The apical electrodes of the array record a distinct increase in CM amplitude while the remaining electrode outputs drop drastically with increase in SPL. Concomitant to the increasing amplitude in the apical part of the array there is a clear, growing dominance of apical phase values over the phase pattern.

Two-tone interaction

It is a well known fact that an intense tone decreases the CM amplitude of a simultaneously presented second tone (Wever et al., 1940). It is however a less well known fact that a high frequency tone is more effective in causing a reduction in the CM of an equally intense low frequency tone than the reverse. Although there are several indications to that end in the literature this property of two-tone interaction has never received much attention (see Wever et al., 1940, p. 271, Fig. 4; Nieder & Nieder, 1968 a, 1968 b). Traditionally two-tone interaction studies were carried out with one recording electrode on the round window membrane or with a set of widely spaced electrodes along the cochlear partition. It is the purpose of this section to demonstrate that the multi-electrode technique is very useful for the investigation of this phenomenon because it provides—unlike the traditional techniques—information about the shape variation of a given CM pattern in the presence of a second tone. One would expect that in response to a bitonal stimulus interaction would occur in the region of the basilar membrane where the excitation patterns of the two single stimuli overlap. It is here that hair-cell outputs of one stimulus are modified due to the presence of the other stimulus, provided, that is, that the system is driven in the nonlinear part of the dynamic range. Thus tonal interaction is essentially a local phenomenon and the hair-cell output pattern of the first stimulus is affected only

in those parts where it overlaps with the output pattern of the second stimulus.

In Fig. 3.19.A a typical example of the mutual effect of two tonal stimuli on their respective CM amplitude distributions is given, the SPL of both stimuli was 90 db the fre quencies being 13 kHz and 8 kHz respectively Despite the fact that the CM amplitude of the 13 kHz pattern is much smaller than that of the 8 kHz pattern, especially in the apical part of the array it is the 13 kHz tone which causes a drastic reduction in the 8 kHz pat tern, while the 8 kHz tone has very little effect on the 13 kHz pattern. Thus the higher fre quency stimulus is more effective in influenc ing the CM pattern of the lower frequency stimulus in the basal turn than the reverse. For the interpretation of this observation it would certainly be misleading to judge the magnitude of the local mechanical stimuli activating the hair-cell generators directly on the basis of the CM amplitude at any one elec trode out of the array Since the 13 kHz CM is smaller in amplitude one would have to argue that the locally 'smaller 13 kHz stimulus is more effective in tonal interaction than the locally larger 8 kHz stimulus This, of course, is not the case and Fig. 3.19.A clearly demon strates that the CM amplitude at any one electrode is not a direct measure of the ampli tude of the corresponding local mechanical stimulus applied to the membrane. The CM pattern is the spatially filtered image of the hair-cell output distribution the CM amplitude in the 13 kHz pattern is smaller than that of the 8 kHz pattern due to the shorter wave lengths of the excitation pattern and the consequent stronger phase cancellation effect.

The data in Fig. 3.19.A can be explained by reference to the work of Engebretson & Eldredge (1968). These authors studied the proper ties of an electrical analogue model with peak clipping intended to mimic the nonlinear characteristics of a unit generator along the membrane. A particularly interesting feature of their model was the greater *effectiveness* of a larger stimulus in reducing the response due

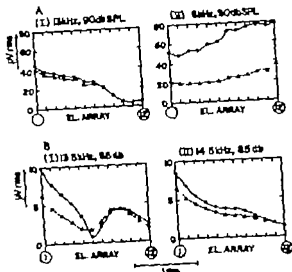


Fig. 3.19 (A) Typical example of mutual influence of two tones on their respective CM amplitude distributions. The 8 kHz tone has very little effect on the 13 kHz CM pattern, the 13 kHz tone, however causes drastic reduction in CM response of the 8 kHz tone. (A I): ●—● 13 kHz (90 db) pattern; ▲—▲, 13 kHz (90 db) pattern in the presence of 8 kHz (90 db). (A II): ●—● 8 kHz (90 db) pattern, ▲—▲ 8 kHz (90 db) pattern in the presence of 13 kHz (90 db).

(B) Typical interaction pattern in the case of two tones with little difference in frequency i.e. nearly 'co-extensive' excitation distributions. (A and B were obtained from different guinea pigs.) The mutual influence of the two tones on their respective CM amplitude patterns is nearly equal. (B.I): ●—● 13.5 kHz (85 db) pattern; ▲—▲ 13.5 kHz (85 db) pattern in the presence of 14.5 (85 db). (B.II): ●—● 14.5 kHz (85 db) pattern; ▲—▲ 14.5 kHz (85 db) pattern in the presence of 13.5 kHz (85 db).

to a weaker stimulus; the weaker stimulus had only very little *effect* on the output of the stronger stimulus. This *feature* applies to the situation in Fig. 3.19.A. According to Greenwood's function (G_1) maximum displacement occurs at 13 kHz in the region around electrodes 8 and 9 maximum displacement of the 8 kHz *excitation* pattern, however occurs apical to the array position. Thus the 13 kHz stimulus is locally (that is in the region of the electrode array) stronger than the 8 kHz *stimulus*. It is therefore not surprising to find a relatively stronger influence of the 13 kHz

tone on the 8 kHz CM pattern than the reverse.

One would predict on the basis of the analogue model that two tones whose excitation patterns overlap each other almost entirely would exert very nearly equal mutual influence on their respective CM patterns. This case is illustrated in the typical example of Fig. 3.19 B. Both stimuli (13.5 kHz and 14.5 kHz) were equally intense (85 db). It can be seen that both CM amplitude patterns are affected to an almost equal degree by tonal interaction.

The examples given clearly indicate that tonal interaction is a local phenomenon depending on the extent to which two interacting excitation patterns overlap. The multi-electrode technique providing information about the spatially graded influence of interacting stimuli may prove useful in the study of this and other related nonlinear phenomena.

Tonal overstimulation

One may speculate that the recording of electrical signals from the cochlea is potentially a more sensitive method of assessing experimentally induced local hair-cell damage than the conventional histological techniques. This could be so because loss of hair-cell activity may precede histologically verifiable damage. In theory there exists the possibility to reconstruct from electrical recordings the extent of activity loss. Let $CM_B(x)$ and $CM_A(x)$ be the CM patterns measured before (B) and after (A) application of a traumatic stimulus S. Imagine that B and A were obtained for the same test stimulus. According to equation (1 D) the difference between the two CM patterns, $\Delta CM_{BA}(x)$ is the spatially filtered image of the difference between the corresponding hair-cell output distributions, $\Delta H_{BA}(x)$:

$$\Delta CM_{BA}(x) = \Delta H_{BA}(x) W * (x) \qquad (1\,D)$$

with the inverse spatial filter operation $\Delta H_{BA}(x)$ can be written in the form

$$\Delta H_{BA}(x) = \frac{\lambda}{2}\Delta CM_{BA}(x) - \frac{1}{2\lambda}\Delta CM''_{BA}(x) \qquad (1\,E)$$

$\Delta H_{BA}(x)$ is the hair-cell activity loss due to the effect of the traumatic stimulus S and theoretically speaking it can be reconstructed according to equation (1 E) provided $\Delta CM_{BA}(x)$ is known from the experiment. However in reality one faces the problem whether the weighting function $W(x)$ was affected by the application of S, i.e. whether the signal conduction properties of the cochlea were altered; for instance one could imagine that the collapse of hair cells changes the conditions for the signal spread along the canals. In cases where S influences the weighting function equation (1 E) is not valid because it requires

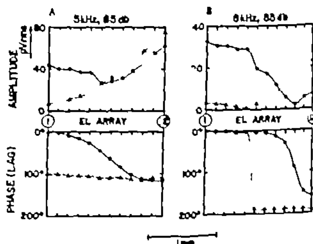


Fig. 3.20. Effect of tonal overstimulation (exposure tone 13 kHz, 130 db, 3 min) on the CM amplitude and phase patterns of two test tones 5 kHz, 85 db (A) and 8 kHz 85 db (B). (A and B obtained in the same guinea pig.) ●—● Pattern before overstimulation with 13 kHz tone. ▲—▲ pattern after overstimulation with 13 kHz tone. The phase values in the post-traumatic situation are referred to that of 1 in the pretraumatic situation.

(A) Relative decrease of amplitude in the basal, relative increase in the apical part of the array. Shift in basal phase values towards apical phase values. This points to the dominant influence of large-phase-lag contributions from unimpaired apical hair-cell generators.

(B) Shape variation similar to that in (A). Note the disappearance of the amplitude minimum at 10 after overstimulation. The persistence of basal phase values indicates residual hair-cell activity in the basal part of the hair-cell output pattern.

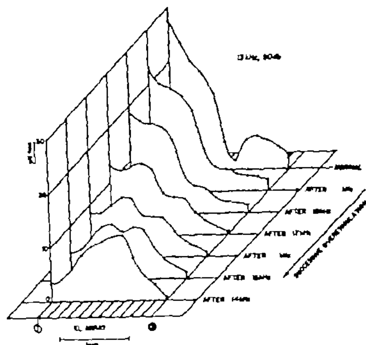


Fig. 3.21 CM amplitude pattern of test tone, 13 kHz, 90 db, shown after successive overstimulation with exposure tones. The pre-traumatic pattern is marked NORMAL. Each exposure tone was applied at 130 db for 3 min. The CM pattern AFTER 21 kHz exposure tone, AFTER 21 20, 19 kHz exposure tones etc. is displayed. Thus AFTER 14 kHz denotes the 13 kHz (90 db) CM pattern obtained after successive overstimulation with 21 20 19 ... 15 14 kHz. Notice the amplitude minimum at 7 in the NORMAL pattern, this minimum disappears upon over stimulation. Note the relative decrease in amplitude *in the basal part of the array* which contrasts well with the relative increase around electrodes 7 8, 9

equal weighting functions for the pre- and post-traumatic situations. Thus for any one traumatic stimulus S it is necessary to establish the constancy of $W(x)$ before equation (1 D) can be applied with confidence. Clearly this can only be achieved by a large series of experiments using graded traumatic stimuli S and complementary histological inspections. Since these investigations were beyond the scope of the present project we did not pursue the matter to the desirable depth. Nevertheless, we did perform some related experiments and the results indicate the suitability of the multi-electrode technique in the study of experimentally induced, local hair-cell activity loss. Moreover the results fully conform to the concept of spatial filtering and this is the main reason why some examples are given here.

Fig. 3.20 displays the results obtained in an experiment concerned with tonal overstimulation The CM amplitude and phase patterns of a 5 kHz and 8 kHz test tone (85 db) were measured before and after overstimulating the guinea pig's ear with a 13 kHz tone of 130 db for 3 min. The 13 kHz exposure tone causes maximum disturbance (according to Greenwood's formula G_1) in the region around electrodes 8 and 9 and it is probably correct to assume that hair-cell activity loss results especially in this and the more basal region due to the flat basal slope of the excitation pattern. Thus, as far as the 5 kHz and 8 kHz test excitation patterns are concerned, we would expect a reduction in hair-cell output in the basal parts of their hair-cell output patterns. This expectation is confirmed by the results in Fig. 3.20. The 5 kHz pattern shows a marked decrease in CM amplitude in the basal part of the array while the amplitude shows a relative increase in the apical part. It is interesting to note that in the 5 kHz phase pattern phase values shift towards the apical phases indicating the dominating influence of large-phase lag contributions from unimpaired hair-cell generators. Essentially similar shape variation of the CM pattern occurs in the 8 kHz case. However the persistence of basal phase values demonstrates residual hair-cell activity in the basal region of the hair-cell output pattern. The 8 kHz CM amplitude pattern lends strong

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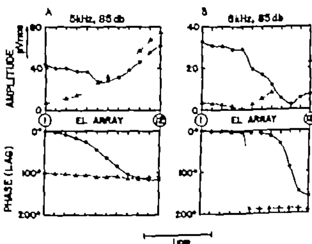


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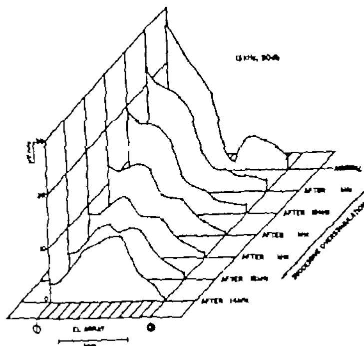


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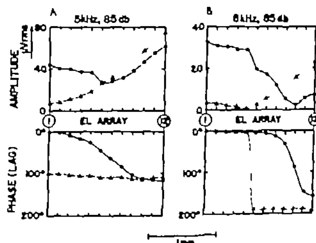


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(B) Shape variation similar to that in (A). Note the disappearance of the amplitude minimum at 10 after overstimulation. The persistence of basal phase values indicates residual hair-cell activity in the basal part of the hair-cell output pattern.

Discussion

It was shown that the spatial filter hypothesis adequately describes the relationship between the hair-cell potential distribution and the CM distribution in the cochlear canals. All salient features of experimental CM patterns measured with the multi-electrode array were found to be explicable on the basis of the spatially filtered excitation pattern along the membrane. Our experimental data provide evidence for an electrical excitation pattern $H(x)^*$ whose shape is in agreement with recent measurements of the mechanical behaviour of the basilar membrane in the high frequency region. The shape of $H(x)^*$ suggests a frequency response curve with a high frequency slope of approximately 110 db per octave and a low frequency slope of approximately 10 db per octave. This compares favourably with the results obtained with the Mössbauer technique at an SPL of 90 db. In addition our electrical data complement mechanical measurements in that they provide evidence for the tuning of the membrane at stimulus intensities lower than 90 db where mechanical data are not available. The insignificant shape variation of the CM pattern up to an SPL of 80 to 90 db indicates the uniformity of the mechanical tuning characteristics over this part of the dynamic range.

Von Békésy's studies in the more apical region of the basilar membrane yielded response curves with high frequency slopes distinctly flatter than the ones suggested by the response curves obtained in the basal part of the membrane. However, von Békésy (1960 a, p. 504) noticed in his records the tendency for a greater sharpness with increasing best frequency of the response curves. Given a systematic tendency for greater sharpness, a 'progression in tuning' (Johnstone 1969) from apex to base along the membrane, especially with respect to the high frequency slope, the discrepancy between the recent basal data and von Békésy's more apical data could be resolved. At the present it is impossible to decide this point because direct observation from the intermediate frequency region is lacking and thus a 'progression in tuning' is not substantiated experimentally. One may question whether a progression in tuning would explain the discrepancy between the results completely; it could well be that the different techniques used account to some extent for the difference between the data. Rhode & Geisler (1969) measured high frequency slopes of 90–150 db per octave with the Mössbauer technique in the squirrel monkey; they obtained their results in the 6 to 9 kHz region of the basilar membrane. Von Békésy performed his optical studies up to frequencies of 4 kHz but never did he report of slope values in this order of magnitude. Unfortunately direct comparison is impossible because von Békésy's results do not refer to the squirrel monkey; thus species differences may partly account for differing results. Nevertheless it seems improbable that progression in tuning is the only satisfactory explanation and the techniques employed may be at least partly responsible for the discrepancy between results. The greater resolution of the Mössbauer technique allows the study to be carried out at the moderate SPL of 90 db. Von Békésy worked on post-mortem preparations at probably much higher sound pressures. It is not unlikely that the steep frequency slope is particularly sensitive to slight post-mortem deterioration of inner ear tissues. One could also suspect that the mechanical frequency response curves change shape when sound pressure increases beyond 90 db. However the latter argument does not seem to apply since von Békésy reported linearity of the mechanical vibrations up to SPLs of 130 to 140 db.

support for the spatial filter hypothesis. In the pretraumatic situation electrode 10 records a distinct amplitude minimum in the post traumatic situation however this minimum is lost and apical electrodes show a relative increase in amplitude. This implies, of course, that the pretraumatic amplitude minimum is the result of phase cancellation between contributions from out-of-phase hair-cell generators—it certainly does not indicate a local amplitude minimum in the corresponding hair-cell output pattern. Overstimulation with 13 kHz causes a relative decrease of basal contributions and consequently apical contributions dominate the shape of the 8 kHz CM pattern It is obvious that consideration of the incremental change in the output of any one electrode from the array does not give anything but a very crude idea of events happening in tonal overstimulation It is the shape variation of the CM patterns $\Delta CM_{BA}(x)$ which contains the information about the underlying spatially graded reduction in hair-cell activity $\Delta H_{BA}(x)$

Another example is given in Fig 3.21 Here the test tone had a frequency of 13 kHz and an SPL of 90 db The pretraumatic 13 kHz CM pattern is marked NORMAL in the diagram. In the normal CM amplitude pattern we observe a distinct amplitude minimum at electrode 7 The remaining amplitude patterns were obtained for the same test stimulus (13 kHz, 90 db) the progressive shape variation being due to successive overstimulation of the guinea pig's ear with a series of exposure tones. In 1 kHz-steps the frequency of the exposure tone was changed from 21 kHz to 14 kHz. Each exposure tone was presented for 3 min at an intensity of 130 db The 13 kHz test pattern was measured after each presentation and in Fig. 3.21 some of the test patterns obtained are shown (The marking AFTER 14 kHz means: 13 kHz pattern after successive overstimulation with 21 20, 19 18 15 14 kHz) As the frequency of the exposure tone is decreased one would predict a progressive reduction of hair-cell outputs of the 13 kHz pattern moreover one would expect this reduction to be most prominent in the basal part of the 13 kHz hair cell output pattern Thus with successive overstimulation contributions from the basal part of the hair-cell output distribution decrease relative to the contributions from the central and apical part According to Greenwood's function (G_1) maximum displacement occurs at 13 kHz in the region around electrodes 8 and 9 Hence we expect to find a relative increase in CM amplitude in this region and a relative decrease in the more basal region of the array This tendency is well shown by the systematic shape variation of the 13 kHz CM pattern with successive overstimulation. The amplitude minimum in the normal CM pattern is definitely due to phase-cancellation it disappears when the balance of out-of phase contributions is altered by overstimulation

A particularly interesting, though puzzling feature of Fig. 3 21 is the smooth ripple pattern on top of the CM amplitude distributions. This ripple pattern certainly has not a sporadic character and one would hesitate to classify it as meaningless ripple noise However what it means is impossible to decide on the basis of CM recordings alone

ings in the cochlear walls would interfere with the steep resonance properties of the inner ear it would be possible to disqualify me chano-electric data entirely and the only acceptable data left would be those from primary nerve fibres. Such a situation, however does not appear to exist. The fact that the Mössbauer technique and the multi-electrode technique—both of which require the exposure of the basal scala tympani—do suggest high frequency slopes in the order of 100 db per octave or more is a clear indication that the 'steepness' of the tuning characteristics is retained despite openings. The decisive experiment, however was done by Evans (1970 *b*) He monitored tuning curves from primary neurones with high characteristic frequency before and after exposure of the basal scala tympani in the guinea pig. He found no change in the neural tuning curves after exposing scala tympani. This is convincing evidence for the negligible influence of openings in the cochlear wall on the tuning properties of the inner ear

At the present it seems necessary to postulate a sharpening mechanism intervening between the mechano-electric input of the cochlear transducer and the neural output. Such a sharpening mechanism will be operating mainly as regards the low frequency slope of the neural tuning curves. Johnstone (1969) suggested slope detection as such a possible mechanism. According to his view it is the steep apical slope of the mechanical excitation pattern with the associated large phase gradient which could constitute the adequate stimulus for nerve excitation. Although a slope detector mechanism—that is a detector for the first spatial derivative of the excitation pattern—could be the answer to the problem it nevertheless remains an open question whether such a mode of operation would be satisfactory as other functions of the peripheral transduction are concerned. Taking the first spatial derivative of a given excitation pattern means rarefying the energy mechanically available—an operation inherently unlikely when considering the minute amount of acoustic energy which suffices to produce auditory sensation at threshold. The fibres of the external spiral bundle with their multiple innervation of hair cells appear to be well suited for spatial summation, that is spatial integration, rather than spatial differentiation.

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In view of the enormous technical difficulties of any conceivable direct experimental study of a sharpening mechanism it is probably wise to adopt a somewhat reluctant attitude towards the acceptance of the flat low frequency slope of mechanical tuning curves, the slope which of course calls for such a sharpening mechanism. Could it not be that the basilar membrane is sharply tuned, that it is a juxtaposition of individual resonator elements; could it not be that there is little damp-

It would be interesting to know whether the size of the Mössbauer probe influences the results, especially the value for the steep high frequency slope. There could well be a maximum slope value recordable with a Mössbauer source of a given size in other words it needs to be clarified whether the size of the Mössbauer source imposes certain limitations on the maximum slope values obtainable. Furthermore the slight deviation of the results measured with the Mössbauer technique from Greenwood's *function* (G_1) for the locus of maximum displacement seems to indicate that the Mössbauer probe loads the vibrating tissues thereby reducing their best frequencies slightly to lower values. It appears that a series of experiments is called for with Mössbauer probes of different sizes. Such experiments could furnish the criteria for the assessment of the limitations of the data measured with the Mössbauer technique.

Likewise objections may be raised as regards the high frequency slope value of 110 db per octave suggested by our function "$H(x)$". It must be remembered that "$H(x)$" is the result of a fitting procedure: we assumed hypothetical functions $H(x)$ and we studied the fit between the corresponding hypothetical CM distribution and our experimental CM patterns. $H(x)$ comprises an amplitude and a phase function and it is the combination of these two functions which is responsible for the resulting shape of the corresponding theoretical CM distribution. Thus, in theory slight modifications of the envelope (amplitude) function of "$H(x)$" can be compensated by modifications of the phase function of "$H(x)$" and the resulting CM distribution may appear unchanged. It is therefore obvious that the value of 110 db per octave indicates only the order of magnitude of the high frequency slope—it is in the strict mathematical sense not binding. Theoretically speaking functions $H(x)$ other than the one ("$H(x)$") arrived at by our graphical fitting procedure, could exist, whose spatially filtered image could conform to experimental CM patterns. In the attempt to establish the unique hair-cell *output function* we inverted the spatial filter operation. This inversion, however *raised* additional problems because it exposed the strong influence of spatial ripple noise on the direct analytical deduction of $H(x)$ *from* experimental CM patterns. Although "$H(x)$" is in accordance with mechanical data and therefore *most probably* a correct description of the excitation pattern along the membrane we could not *prove* in this study that it is the *only possible* description satisfying our CM data. Hence the question of uniqueness is *left* unsolved.

The study of the excitation pattern along the membrane via the CM potential distribution along the cochlear ducts is basically an indirect method. The CM pattern is the spatially filtered image, the smeared out version of the electrical excitation pattern. In any case a deductive step is necessary for the recovery of the original excitation pattern from the experimentally accessible CM distribution. The fact that it is an indirect method is accentuated by the problem of spatial ripple noise. The smooth ripple pattern consistently seen in experimental CM distributions hinders the direct deduction of $H(x)$ from CM data and the question of the origin of the ripple structure is raised. This circumstance exposes the shortcomings of the electrical method in the exploration of the excitation pattern.

The major question raised by the studies of the mechano-electric input of the cochlear transducer is not so much concerned with the high frequency slope of the response curves, it is rather that of the flat low frequency slope. The asymmetric shape of the mechanical tuning curves which according to our data seems to apply also at SPLs lower than 90 db is at complete variance with the more nearly symmetric shape of tuning curves from first order neurones at low SPLs. There is no way out from this discrepancy via the route suggested recently by Huxley (1969). Huxley doubted the reliability of results obtained from inner ears which had their canal walls opened for experimental access. If it was true that open

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ing in the mechanical transducer? Pumphrey & Gold (1948) (Gold & Pumphrey 1947/48) felt that they had convincing, unambiguous psychoacoustic evidence for the 'resonance hypothesis. In their experiment listeners were asked to distinguish between two series of tone pulses. Each pulse consisted of n periods of a 5 kHz tone the silent interval between pulses measured m periods of the 5 kHz tone Let us call a tone pulse which begins with a positive half wave A and that with a negative half wave B Distinction had to be made between the series AAA and the series ABA. The Fourier spectra of the two series are different, they become more and more similar with increasing $(m+n)$ Pumphrey & Gold found that listeners were perfectly capable to distinguish the two series provided the silent interval between pulses was less than $m=30$ periods. For m greater than 30 discriminability became progressively worse and ultimately faded away completely Pumphrey & Gold based their interpretation of the experiment on the temporal aspects of the stimulus signals rather than the difference in the frequency spectra Since the only difference between tone pulses A and B is a 180 phase shift they argued that some kind of phase memory' must exist in the mechanical transducer For ABA to be distinguishable from AAA they reasoned the mechanics of the ear must allow the interaction between a preceding pulse A with the following pulse B or A respectively This implied very little mechanical damping so that the long lasting afteroscillations from the previous pulse could interact with the negative or positive going onset of the following pulse Since discrimination is possible for silent intervals with m up to 30 Pumphrey & Gold concluded that the basilar membrane is extremely lightly damped or what is equivalent that it is extremely sharply tuned The argument would certainly be valid if as Pumphrey & Gold have implicitly assumed the mechanical interaction had to take place in a 5 kHz resonator' This assumption however is false The inner ear is a distributed system not just one single resonator The afteroscillations of a preceding pulse need not interact with the following pulse at one point along the membrane; discrimination seems possible as long as offset transients of the preceding pulse are present on the membrane at the onset of the following pulse Surprisingly it has been constantly overlooked in the past (von Békésy & Rosenblith, 1951 Huxley 1969) that the experimental finding of Pumphrey & Gold is in perfect agreement with von Békésy's direct observations of the basilar membrane vibration which revealed strong damping. According to von Békésy s measurement in the human cochlea a transient disturbance takes about 5 msec to travel from base to apex. However $m=30$ periods of a 5 kHz tone equate to $30\times0.2=$ 6 msec this is the duration of the silent interval between successive tonal pulses for which discriminability starts to deteriorate. Thus it seems that the strongly damped basilar membrane is able to provide the spatial cues for the discrimination required in the experiment of Pumphrey & Gold. It certainly does not follow that this experiment constitutes a valid proof that the resonance hypothesis is true

The apparent necessity to postulate a 'sharpening mechanism must provoke the study of the transduction process itself a study which is essential for the elucidation of the causal chain of linear and nonlinear events leading from mechanical displacement to production of neural spikes. In this investigation we used the electrical potential in the cochlea as an index of mechanical excitation without touching the question of the rule of the potential in eliciting neural impulses. As regards this question opinions vary widely Tasaki (1957) suggested 'that the generation of microphonics appears to be an essential intermediate step intervening between the mechanical motion and initiation of nerve impulses On the other hand Dallos (1969 b) felt that CM potentials might not necessarily form an essential link in the peripheral transduction chain Clearly there seems to be no escape from looking at the transduction itself

Summary

The multi-electrode technique was developed for the study of the potential distribution in the basal 1/2 turn of scala tympani along the basilar membrane in the guinea pig's cochlea.

The salient features of the CM distributions in response to sinusoidal stimuli were found to be consistent with the concept of spatial filtering. According to this concept the CM distribution has to be considered as the spatially filtered image of the electrical activity along the organ of Corti. Various hypothetical activity patterns were tested by comparing their corresponding theoretical CM patterns with experimental CM data. The best fitting activity pattern was compatible with recent observations of the mechanical behaviour of the basal end of the basilar membrane. Inversion of the spatial filter operation, although possible in theory met with difficulties in practice. The significance of slight ripples in experimental CM patterns is obscure. The direct, analytic deduction of the electrical excitation pattern from the experimental CM pattern must await the clarification of the rôle of 'ripple noise'.

The multi-electrode technique is potentially a useful tool in studies concerned with local modifications of a given electrical activity pattern. Local changes in electrical output cause a variation in the shape of the corresponding CM pattern. This was exemplified in the case of the differential growth of the activity pattern in the nonlinear range, and the cases of two-tone interaction and tonal overstimulation.

Zusammenfassung

Zur Untersuchung der Potentialverteilung entlang der Basilarmembrane wurde eine Sonde eng gesetzter Elektroden verwendet.

Die Eigenschaften der gemessenen Verteilungen bestätigen die Hypothese derzufolge die Potentialverteilung als die örtlich gefilterte Version der elektrischen Aktivität im Cortischen Organ aufgefasst werden muss. Um Aufschluss über die Reizverteilung entlang der Basilarmembran zu erhalten, wurden verschiedene hypothetische Reizverteilungen angenommen und ihre entsprechenden gefilterten Abbilder mit gemessenen Kurven verglichen. Diejenige Reizverteilung, die den Messkurven gerecht wurde, ergab zugleich eine gute Übereinstimmung mit dem in jüngster Zeit bekannt gewordenen mechanischen Verhalten der Basilarmembran am basalen Ende. — Zur direkten Ableitung der Reizverteilung von gemessenen Potentialkurven wurde die Ortsfilterfunktion invertiert. Diese Operation ist theoretisch möglich, jedoch bereitet die Inversion beträchtliche praktische Schwierigkeiten auf Grund der Unregelmässigkeiten kurzer Wellenlänge, die in den Messkurven vorliegen.

Die Elektrodensatzmethode ist besonders nützlich für Untersuchungen, die sich mit lokalen Änderungen der Reizverteilung befassen. Solche Änderungen modifizieren die Form der messbaren Potentialverteilung. Dies wurde gezeigt in den Fällen der Potentialzunahme im nichtlinearen Bereich, der Wechselwirkung zweier Töne und im Falle tonaler Überreizung.

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Résumé

Une technique faisant appel à une dispositif d enregistrement utilisant des electrodes multiples a été mis au point afin d étudier la répartition du potentiel microphonique dans la spire basale de la cochlée

Les caractéristiques de la répartition du potentiel microphonique provoqué par des sons sinusoïdaux sont au bon accord avec le principe de filtrage spatial. Selon ce principe on doit considérer que la répartition du potentiel microphonique représente l image de l activité électrique de l organe de Corti modifiée par un filtrage spatial Plusieurs répartitions hypothétiques ont étés proposées et confrontées avec les données experimentales concernant le potentiel microphonique. La répartition que présentait le meilleur accord avec les données récentes sur la mécanique cochléaire a été retenue L inversion du mécanisme du filtrage spatial possible en théorie, s est révelé difficilement réalisable. La signification de petites ondulations sur les graphiques representant la répartition spatiale du potentiel microphonique est difficile à expliquer

La technique des electrodes multiples apparait comme un instrument utile dans toutes les recherches consistant a décrire la répartition spatiale d une activité électrique particulière. Des changements affectant certaines sources, assez localisées, de potentiel entraine des variations importantes du pattern global du potentiel microphonique Cet effet est clairement démontré par l observation des différences dans l augmentation d amplitude du potentiel microphonique en fonction de l intensité en certains endroits, principalement dans la zone de nonlinéarité, ainsi que dans le cas des interactions entre deux fréquences, et aussi en présence d intensités excessives.

Acknowledgments

I am most grateful to Dr I C Whitfield, Director of the Neurocommunications Research Unit University of Birmingham, for his encouragement, guidance and valuable criticism throughout the course of this study

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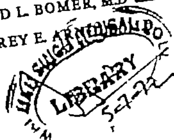
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CONTENTS

This study was supported by Public Health Service
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Classification and Incidence

This is the third in a series of rare tumors of the head and neck seen at this University Hospital. Moore and Arnold (47) reported 14 cases of rare tumors of the head and neck, both benign and malignant. Day and Arnold (21) reported 15 further cases of rare benign head and neck tumors. During the academic three year period 1966–1969 15 additional cases of rare malignant tumors were observed as reviewed in this paper. All of the cases were seen and treated by the Division of Otolaryngology except two. Case 8 was seen in consultation with the General Surgery Service. Case 15 was treated jointly with the Division of Plastic Surgery. In contrast to the previous publications, the patients were compiled from the University Hospital Outpatient Department (8), the Veterans Administration Hospital (3), and the private practice of the attending staff (4). This varied source may explain certain differences of distribution (age, sex, race) as compared to series I and II. The definition of a rare tumor was the same as discussed previously (47, 21).

The statistics of clinic visits, hospital admissions, and operations are listed in Table I. As was pointed out by Day and Arnold (21) there is an unusually high frequency of rare tumors for the relatively small number of total admissions to our service.

The rare malignant tumors are presented by location and type of humor. Table II summarizes the cases, including the tissue diagnosis, location, age, gender of patient, treatment, and results.

Table III compares this series with series I and II. As would be expected with malignant tumors, most of the patients were in the fifth, sixth, and seventh decades of life. In contrast to our two previous series, there is an equal distribution between white and Negro patients, which agrees with the ratio of white to Negro patients seen in our clinic.

Cancer of the Nasal Septum

Cancer of the nasal septum is a rare disease. According to Martin (42), malignant tumors of the nasal cavity and the paranasal sinuses comprise 0.2% of all cancer and 3% of cancer of the upper respiratory and alimentary tract. Nasal septal tumors are usually of the epithelial variety (48). Tumors presenting in the nasal cavity often are secondary to neoplasms of the ethmoid and maxillary sinuses, nasopharynx, orbit and external skin. With the exception of allergic and benign polyps, more than one half of the intranasal neoplasms are malignant.

The signs and symptoms of nasal malignancies vary and are very similar to many benign processes (9). Therefore, they are often overlooked in the early stages and treated conservatively. Malignant lesions are usually friable, granular, infiltrating, and bleed easily. Besides epistaxis there may be nasal obstruc

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Table I *Hospital statistics 1964–1969*

	1964–1965	1965–1966	1966–1967	1967–1968	1968–1969	Total
Total OPD visits	52 599	60 403	64 717	65 322	66 840	309 881
ENT clinic visits	1 218	1 398	1 656	2 004	2 120	8 396
Admissions	12 380	11 891	11 490	12 013	13 384	61 158
ENT admissions	314	322	482	458	521	2 097
Total operations	5 945	6 255	6 110	6 388	7 124	31 822
ENT operations	427	340	488	457	485	2 197

Table II *Summary of tumors observed*

No	Tumor	Location	Race and Sex	Age	Treatment	Recurrence
1	Squamous cell carcinoma	Nasal septum	WM	63	Cobalt surgery	None for 2 / years
2	Malignant mixed tumor	Nasal septum	WF	60	Surgery	None for 3 years
3	Malignant melanoma	Nasal septum	WM	54	Surgery	None for 6 months
4	Angiosarcoma	Maxillary and ethmoid sinuses	NF	47	Cobalt surgery	Died of disease
5	Rhabdomyosarcoma	Ethmoids, antrum and orbit	WF	1 /	Cobalt surgery	None for 8 months
6	Adenocarcinoma	Parotid gland	NM	44	Surgery	None for 12 months
7	Mucoepidermoid carcinoma	Parotid gland	NF	18	Radiotherapy surgery	Local recurrence 21 months
8	Malignant mixed tumor	Submaxillary gland	NF	21	Surgery cobalt	Died 2 days post op
9	Mucoepidermoid carcinoma	Bilateral submaxillary glands	WM	63	Surgery chemotherapy	Died of disease after 15 months
10	Adenoid cystic carcinoma	Nasopharynx	NM	52	Surgery	None for 3 years
11	Carcinoma of minor salivary gland	Ventricular fold	WM	64	Surgery	None for 4 years
12	Papillary adenocarcinoma	Tongue	WM	83	Radiation, surgery	None for 2 years
13	Adenoid cystic carcinoma	Ear canal	NM	48	Cobalt, surgery	Died of disease
14	Ceruminoma	Ear canal	NF	50	Surgery cobalt	In nasopharynx 7 months later
15	Malignant melanoma	Hard palate	NM	62	Surgery immunotherapy	Alive with lung metastasis at 6 months

tion pain secondary infection external deformity cervical metastasis, serous otitis, tearing, and hyposmia. A variety of other signs and symptoms may develop with extension of the tumor into adjacent areas, viz. orbit, cranium sinuses, etc

There is a remarkable variety of neoplasms arising in the nasal cavity and paranasal sinuses. (34) The squamous cell carcinoma, the most common presumably arises following squamous cell metaplasia Most of these occur anteriorly or near the mucocutaneous

Table III. *Lesions, sex, race age*

	First series (benign)	First series (malignant)	Second series (benign)	Third series (malignant)	Total
No. of rare cases	9	5	15	15	44
Types of lesions	7	4	10	11	32
Benign	9	—	15	—	24
Malignant	—	5	—	15	20
Male	3	2	6	9	20
Female	6	3	9	6	24
White	1	1	1	7	10
Negro	8	4	14	8	34
Ages					
0–10	—	1	7	1	9
11 20	4	1	3	1	9
21 30		—	—	1	1
31–40	2	1	1	—	4
41 50	2	—	1	4	7
51–60	1	—	2	3	6
61 70	—	2	1	4	7
71–80	—	—	—	—	—
81 90	—	—	—	1	1

junction. Deutsch (22) reported one case and reviewed the literature, finding 27 cases of squamous cell carcinoma of the nasal septum. Lyons (37) reported seven cases of squamous cell carcinoma of the septum. Martin and Headorffer (43) reviewed the literature on malignant mixed tumors of the nasal septum. Basal cell carcinoma arises from the vestibular skin Adenocarcinoma, fibrosarcoma, lymphoepithelioma, osteogenic sarcoma, and chondrosarcoma are also seen. Occasionally the nose is the site of metastatic carcinoma.

The success of the treatment is directly proportional to the size of the lesion and the differentiation of the malignant cells Large lesions, poorly differentiated tumors, and those with local or distant spread have a poorer prognosis. Early lymphatic metastasis is not a feature of squamous cell carcinoma of the external nose (19 20). Lymphatic spread from an internal nasal tumor tends to be earlier (14) When a lesion is confined to the septum, radical neck dissection should not be performed unless nodes are palpable because nodes may appear on the contralateral side only

Malignant melanoma (19 20, 67) usually originates in the skin of the external nose but rarely occurs in the mucous membrane of the nasal cavity Malignant melanoma of the nasal cavities and paranasal sinuses is almost invariably primary and constitutes less than 2% of all malignant melanomas (67). Malignant melanomas of the nose usually spread to regional lymph nodes. Distant metastasis occurs late, which is somewhat different from the melanomas of the skin elsewhere in the body The usual sites in the nasal cavities are the anterio-inferior portions of the septum and the inferior turbinates (26, 52).

Treatment has been most successful with surgery This may be in combination with ir radiation. Large lesions which are inoperable may be palliated with irradiation. Occasionally chemotherapy may be employed. Case 1 was first treated with irradiation of a squamous cell carcinoma which failed to control the disease and radical surgery was then done without evidence of recurrence $2^1/_2$ years later Case 2 was treated only with surgery and is free of disease after 1 $/_2$ years.

Reconstruction of defects resulting from excision of carcinomas may be either immediate or delayed. Septal reconstruction often taxes the ingenuity of the surgeon. Saddling and falling of the tip is avoided by the use of a columella strut, or bone from the posterior septum. External defects may be



Fig 1 Waters view shows thickened mucosa of the maxillary sinuses. The bulbous deformity of the nose can be seen (Case 1).

closed with local flaps composite grafts fore head flaps, or tubed pedicle flaps A prosthesis can be used during the early post operative period and in certain other cases permanently

Case 1 A white man age 63 years, was referred to the ENT clinic after a biopsy from a mass in the left nasal vestibule by his local physician indicated squamous cell carcinoma. There had been a three month history of crusting ulceration and bleeding from the left nasal cavity A progressive enlargement of the tip of the nose had been noticed for about one year The patient also had paresis of his legs due to poisoning from excessive rum intake

ENT examination showed a marked enlargement and bluish discoloration of the external nose. A friable mass was present in the left nasal vestibule which arose from the nasal septum and bled freely when palpated. A hard, non tender node (1 × 1 cm) was present in the left submaxillary triangle X ray films showed the nasal mass and bilateral maxillary sinusitis (Fig 1)

The primary lesion was treated with cobalt (5 000 R) and a left radical neck dissection Three months after the cobalt treatment was completed the nasal tip became necrotic and sloughed Biopsy at the base of the nose proved recurrent squamous cell carcinoma Following radical excision of the remaining nose the patient preferred a prosthesis to plastic reconstruction of the nose He is now 2 1/2 years following resection of his nose and there is no local or distant evidence of tumor Fig 2 is a photomicrograph of the tumor

Case 2 A white woman age 60 years, con-



Fig 2 Squamous cell carcinoma of the nasal septum H and E, 100

plained of nasal obstruction Eighteen years prior to this admission she had a nasal septum tumor removed which was diagnosed as misplaced salivary gland tissue She remained asymptomatic for seven years at which time second intranasal procedure was performed Following this operation she remained free of symptoms until six months prior to admission when nasal obstruction recurred

Examination demonstrated a large midline tumor with septal deviation to the left. Paranasal sinus X-ray films showed a soft tissue fullness and distortion slightly to the right of the midline of the nasal septum A biopsy was diagnosed as malignant mixed tumor of the septum The tumor was removed through lateral rhinotomy and removal of the entire septum was necessary The patient is free of recurrence three years post op.

The tumor was composed of embryonal epithelial cells with areas of myxoid chondroid, and adenoid differentiation. The tumor cells invaded the capsule. Because of the aggressive nature and pleomorphism, the tumor was considered to be malignant. Surgical and pathologic details were reported by Martin and Hesdorffer (43).

Case 3 A white man, age 54 years, had noticed a growth on the right nasal septum for about five weeks with slight bleeding but no pain. Prior to referral, a biopsy of the lesion demonstrated malignant melanoma.

Examination showed a lesion 1 cm in dia-



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Angiosarcoma

Angiosarcoma is a rare tumor in the head and neck (38). Bardwil et al. (5) at M. D. Anderson Hospital reported seven cases. A total of 155 cases have been studied in the Laboratory of Surgical Pathology of Columbia University (65). McCarthy and Pack (45) reported 20 cases, while MacComb and Martin (40) saw three cases of the nasal cavity. Most of these reports included the angiosarcoma arising anywhere in the body (24), mainly in various viscera and bones, as well as at any site in the respiratory tract (1, 40). Angiosarcoma afflicts chiefly adults without sex preponderance. The symptomatology depends on the location of the tumor. Bleeding is sometimes severe and may require ligation of major blood vessels.



Fig. 3. Proptosis and right deviation of the right eye caused by an angiosarcoma of the right paranasal sinuses (Case 4).

Angiosarcoma is also called hemangiosarcoma, malignant hemangioendothelioma, angiofibrosarcoma, hemangioblastoma, hemangioendothelioblastoma, and hemangioendothelioma (65). Its histogenesis is obscure, as suggested by the number of its various names. The concept of the so-called benign metastasizing hemangioma is now generally discredited. Since one can distinguish small blood vessels from lymph vessels only by their contents, the presence of blood in some vessels and protein in others suggests that the tumor may be produced from both blood and lymph vessels (5, 62, 65).

Microscopically, conglomerates of atypical capillaries with a marked tendency to frequent anastomosis predominate. These capillaries are lined with swollen, anaplastic endothelioblasts, which are either rounded or elongated and sometimes accumulated in clumps, partly filling the lumen. A reticulum stain will demonstrate the vascular pattern showing the tumor cells inside the delicate reticulum sheath that encloses each vessel.

The clinical course of these tumors varies. There is a strong tendency to recur locally as well as to metastasize. Metastasis usually takes the hematogenous route, but may be lymphatically spread as well. When feasible,

the treatment of choice is complete excision of the tumor Radical neck dissection is done in cases of controlled primary lesion with cervical metastasis. Irradiation occasionally produces a favorable response (27 45 51). Its prognosis again varies, but the largest series of 155 cases (65) reflected mortality of 56% among adults.

Case 4 A Negro woman, age 47 years (Fig. 3) had a two month history of right proptosis, tearing, sinus trouble" bloody nasal discharge on the right, and a lump in the right neck. Weight loss or impairment of vision were denied. The patient had conservative treatment by a local physician for a short period without response before seeking help at the Medical Center

Examination showed right proptosis, bilateral submaxillary lymphadenopathy (more on the right side) but no lesions could be seen intranasally The sinus x ray films demonstrated clouding of the ethmoids and maxillary sinus, and erosion of the inferiomedial wall of the right orbit, all on the right A biopsy from the right maxillary sinus and ethmoid cells was diagnosed as a poorly differentiated carcinoma. Following reticulum stain of the specimen it was diagnosed as an angiosarcoma.

The patient received 5 000 R cobalt radiation to the right orbital and maxillary region during 13 treatments. The proptosis regressed greatly and the cervical lymphadenopathy disappeared. Right radical maxillectomy was well tolerated with uneventful recovery However three months post op. the patient began to lose weight and pulmonary and cervical metastases appeared. Chemotherapy brought no improvement and the patient died a few weeks later Fig. 4 is a photomicrograph of the tumor



Fig 4 The vascular spaces of the angiosarcoma lined with endothelial cells. H and E, 475

Rhabdomyosarcoma

The first series of our rare tumors (47) contained two cases of rhabdomyosarcoma, one in the middle ear of a 2 year old Negro boy and the other in the nasopharynx of a 16 year old Negro girl. Two varieties of rhabdomyosarcoma are recognized. the form found almost exclusively in children, and the adult type almost exclusively in adults. The 474 cases described by Stout (63 65) had this distribution. 236 in the age group below 15 years, and 238 above. Horn and Enterline (33) observed 39 cases with 18 below age 16 and 21 above.

Rhabdomyosarcoma is the most common

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meter on the right septum just behind the mucocutaneous junction It was raised ir regular hard, and had a black center There was no bleeding and no cervical lymphadenopathy The lesion was removed by resecting the nasal septum, columella, and the skin and mucosa from the floor of the nose. Six months later he was free of local recurrence and meta stasis.

Angiosarcoma

Angiosarcoma is a rare tumor in the head and neck (38) Bardwil *et al.* (5) at M. D Anderson Hospital reported seven cases. A total of 155 cases have been studied in the Laboratory of Surgical Pathology of Columbia University (65) McCarthy and Pack (45) reported 20 cases, while MacComb and Martin (40) saw three cases of the nasal cavity Most of these reports included the angiosarcoma arising anywhere in the body (24) mainly in various viscera and bones, as well as at any site in the respiratory tract (1 40) Angiosarcoma afflicts chiefly adults without sex preponderance. The symptomatology depends on the location of the tumor Bleeding is sometimes severe and may require ligation of major blood vessels.



Fig 3 Proptosis and right deviation of the right eye caused by an angiosarcoma of the right paranasal sinuses (Case 4).

Angiosarcoma is also called hemangiosarcoma, malignant hemangioendothelioma, angiofibrosarcoma, hemangioblastoma, hemangioendothelioblastoma, and hemangioendothelioma (65). Its histogenesis is obscure, as suggested by the number of its various names. The concept of the so-called benign metastasizing hemangioma is now generally discredited Since one can distinguish small blood vessels from lymph vessels only by their contents, the presence of blood in some vessels and protein in others suggests that the tumor may be produced from both blood and lymph vessels (5 62 65)

Microscopically conglomerates of atypical capillaries with a marked tendency to frequent anastomosis predominate. These capillaries are lined with swollen anaplastic endothelioblasts, which are either rounded or elongated and sometimes accumulated in clumps, partly filling the lumen A reticulum stain will demonstrate the vascular pattern showing the tumor cells inside the delicate reticulum sheath that encloses each vessel.

The clinical course of these tumors varies. There is a strong tendency to recur locally as well as to metastasize Metastasis usually takes the hematogenous route but may be lymphatically spread as well. When feasible

the treatment of choice is complete excision of the tumor Radical neck dissection is done in cases of controlled primary lesion with cervical metastasis. Irradiation occasionally produces a favorable response (27 45 51) Its prognosis again varies, but the largest series of 155 cases (65) reflected mortality of 56% among adults.

Case 4 A Negro woman, age 47 years (Fig. 3), had a two month history of right proptosis, tearing, "sinus trouble bloody nasal discharge on the right, and a lump in the right neck. Weight loss or impairment of vision were denied. The patient had conservative treatment by a local physician for a short period without response before seeking help at the Medical Center

Examination showed right proptosis, bilateral submaxillary lymphadenopathy (more on the right side) but no lesions could be seen intranasally The sinus x ray films demonstrated clouding of the ethmoids and maxillary sinus, and erosion of the inferiomedial wall of the right orbit, all on the right. A biopsy from the right maxillary sinus and ethmoid cells was diagnosed as a poorly differentiated carcinoma. Following reticulum stain of the specimen it was diagnosed as an angiosarcoma.

The patient received 5 000 R cobalt radiation to the right orbital and maxillary region during 13 treatments. The proptosis regressed greatly and the cervical lymphadenopathy disappeared. Right radical maxillectomy was well tolerated with uneventful recovery However three months post op. the patient began to lose weight and pulmonary and cervical metastases appeared. Chemotherapy brought no improvement and the patient died a few weeks later Fig 4 is a photomicrograph of the tumor



Fig 4 The vascular spaces of the angiosarcoma lined with endothelial cells. H and E, 475

Rhabdomyosarcoma

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Malignant Tumors of the Major Salivary Glands

No tumors of the major salivary gland occurred in either of the two previous reports of rare tumors of the head and neck (21 47). It was observed at the M. D. Anderson Hospital (56) (1944 to 1962), that 4.5% of the total number of patients with malignant tumors of the head and neck had malignant tumors of the salivary glands. Less than 1% of all cancer cases are located in the salivary glands (41). Frazell (29) found 36% of 1751 major salivary gland tumors to be malignant. Among all these tumors, 88% were located in the parotid, and about one third of these were malignant. The submaxillary gland tumors represented only 11.8% of the total, but 53% of these were malignant. Tumors of the sublingual gland numbered only four (0.2%) and all were malignant.

We had four patients with malignant salivary gland tumors: an adenocarcinoma of the parotid gland, a mucoepidermoid carcinoma of the parotid gland, a malignant mixed tumor of the submaxillary gland and a mucoepidermoid carcinoma of both submaxillary glands. There are numerous reports of the first three types of tumors. However we could not find a case report of a mucoepidermoid carcinoma of both submaxillary glands occurring simultaneously. Bilateral occurrence of papillary cystadenoma lymphomatosum (Warthin's tumor) has been reported (70, 15). Frazell (29) had 1 patients with multiple salivary gland tumors in his series of 1751. Of these 16 had bilateral Warthin's tumors in the parotid, and two bilateral mixed tumors in the parotid. Another patient had a benign mixed tumor in one parotid and one submaxillary gland. One patient had bilateral lymphoepithelial lesions of the parotids. The last patient had an acinic carcinoma of the parotid and benign mixed tumor of the submaxillary glands.

In the past there was disagreement concerning the histogenesis and the criteria for histologic classifications. Some of the parotid tumors are still difficult to place in any classification. Work and Gates (69) have outlined a clinically useful classification, dividing the tumors into four groups. (1) epithelial cell origin, (2) supporting tissue origin, (3) local manifestation of constitutional disease, and (4) metastatic disease. The AFIP fascicle by Foote and Frazell (28) is one of the best guides for histologic classification.

The treatment of malignant salivary gland tumors is well described (3 4 8, 19 25 30, 56, 69). It is primarily surgical. Radiotherapy has been employed for palliation of non-resectable tumors, for recurrences, and for post operative cases when the margins contained tumor

The reports of results vary greatly Malignant tumors arising in the submaxillary and



Fig. 6. Case 6, showing right parotid mass which was an adenocystic carcinoma of the parotid.



Fig 5 19 month old girl with lateral displacement of the left eye (Case 5).

malignant head and neck tumor except in the brain (63) Its two types differ also in location In the older age group the primary lesions predominate in the torso and extremities. The primary areas afflicted by the juvenile type involve the head and neck or urogenital tract (61) Both types have a poor prognosis. In the infantile variety the orbit is a typical site where this relatively rare tumor occurs. Porterfield and Zimmerman (55) reported 55 such cases. Local spread proceeds through direct infiltration. Distant metastasis follows the hematogenous and less commonly the lymphatic route Metastases are found in the lungs, lymph nodes, skin subcutaneous tissues, pleura, bones, liver kidney adrenals mediastinum, pericardium pancreas, ovary and brain in that order

The microscopic appearance varies greatly The tumor has been given different names —botryoid embryonal alveolar and pleomorphic—expressing certain gross and microscopic features that characterize these varieties (23 33) The greatest overlap of features concerns the first three varieties and characteristics of each may occur in the same tumor For this reason the term juvenile rhabdomyosarcoma has been proposed (65). The term "pleomorphic is often applied only to the adult type (63)

Treatment of these tumors has not been very successful (64 66) Radiotherapy can be administered preoperatively postoperatively as the sole treatment, or for palliation. When advocated surgery attempts wide resection. However one should be reasonably certain of a cure before performing extensive and mutilating surgery especially in children. Chemotherapy has temporarily produced remissions of local recurrences and metastatic lesions. It may be given to augment the beneficial effects of irradiation Actinomycin D vincristine, and cyclophosphamide (Cytoxan) are the drugs usually administered for this tumor

Case 5 A white girl, age 19 months, was noted to have left orbital swelling and mild, bilateral bloody nasal discharge for one month There was no weight loss, pain or evidence of infection We saw this patient in consultation with ophthalmology and noted lateral displacement of the eye by an orbital mass on the left side (Fig. 5).

Vision and ocular motility were good. Orbital biopsy disclosed the alveolar embryonal type of rhabdomyosarcoma.

The patient received 1800 R cobalt to the left orbital area preoperatively followed by left orbital exenteration left ethmoidectomy and partial maxillectomy The specimen showed post irradiation changes, but some tumor fragments were found. Three days post op a program of chemotherapy was begun with vincristine, actinomycin D and Cytoxan* which had been continued on an outpatient basis. No recurrence nor metastasis was noted eight months later

perineural invasion. The diagnosis was low ...de adenocarcinoma of the parotid gland.

Case 7 A Negro girl, age 18 years, was ...erred to the ENT clinic for evaluation of ...rvical lymphadenopathy Five months pre...ously she had received x-ray treatment by ...r local physician after a tissue diagnosis of ...rcinoma of the skin behind the right ear ...d been made. An enlarging mass behind ...e right ear of five years duration, a three ...ear history of right facial nerve paralysis, ...nd bilateral temporal headaches with occa...ional tinnitus in the right ear had prompted ...er original visit.

Examination showed multiple nodularity in ...he right posterior triangle, mild tenderness ...ver the tail of the parotid gland, and right peripheral facial nerve paralysis. An audiogram and sinus x-ray films were normal.

Total excision of the right parotid gland and right neck dissection were performed. Wide skin excision over the mastoid area, including part of the auricle, was necessary and a split thickness skin graft was applied. Following removal of the specimen, it was noted that the tumor had invaded the jugular foramen. Eight of the 15 lymph nodes contained tumor The tissue diagnosis was mucoepidermoid carcinoma of the parotid gland. The patient received cobalt therapy postoperatively because tumor had remained in the jugular foramen. Twenty-one months postoperatively a recurrence under the right ear was treated with 5-fluorouracil. The chemotherapy was not completed and the patient could not be traced.

Case 8. A Negro woman, age 21 years, had a two year history of a right submandibular mass (Fig. 9) with a period of rapid growth eight months before being seen. She was pregnant during that time and delivered a normal infant two months before being seen. The remainder of the past history and systems review was normal. We saw the patient in consultation with the General Surgery Service for laryngoscopy and nasopharyngoscopy

Examination showed a large, firm, non-



Fig. 9 Malignant mixed tumor of the right submaxillary gland (Case 8).

tender fixed mass in the right submandibular area which displaced the tongue, bulged into the hypopharynx on the right side, and invaded the floor of the mouth on the right. It extended across the midline submentally but there were no palpable nodes in the neck. The tentative diagnosis considered a salivary gland tumor and the history of recent rapid growth suggested a malignant lesion.

At surgery the huge lesion was found to involve the anatomical areas mentioned above. Frozen section of the lesion was reported as malignant tumor involving the submandibular gland. The tumor was incompletely excised at the base of the tongue. The diagnosis from the permanent slides was malignant mixed tumor of the salivary gland. The patient was then treated with cobalt (3000 R) and radical resection of the lesion was completed. She died on the second postoperation day from uncontrollable hemorrhage that could not be anticipated.



Fig Low grade adenocarcinoma of the parotid gland shows invasion of the capsule H and E, 50

sublingual glands are not only more frequent *but also* more aggressive than similar tumors of the parotid gland. The five year survival for the lower grade parotid tumors is about 80% to 85% but drops sharply in the more malignant group. Local recurrence and regional spread to lymph nodes is not uncommon. Some of the tumors, like adenocarcinomas, produce bloodborne metastasis.

Case 6 A Negro man age 44 years, was referred to the ENT clinic because of a mass under the right ear (Fig 6) This mass had been present for about one year and had been completely asymptomatic. The past history was not remarkable.

Examination showed a firm slightly moveable non-tender mass inferior and slightly anterior to the right auricle Saliva from the right parotid duct was clear There was no facial nerve impairment. A tentative diagnosis of a malignant parotid tumor was made because of the rapid growth. A sialogram demonstrated the mass to be fairly discrete and located in the deep portion of the parotid gland.

A total parotidectomy was done with preservation of the facial nerve. The tumor was indeed located in the deep portion of the parotid gland and was very well circumscribed. Some transient paresis of the frontal and zygomatic branches of the facial nerve was noted post-operatively

Figs. 7 and 8 show *the microscopic appearance of the malignant portion of the tumor The tumor had a variable pattern. The* nuclei exhibited variation in size, with some pleomorphism and hyperchromatism. Occasional mitoses were present. The tumor invaded the capsule but the resection margins were free of tumor There was no vascular



Fig 8 Adenocarcinoma of the right parotid gland shows pleomorphism and hyperchromatism. Rare mitosis and cystic structure can be seen. H and E, 475

mors in the buccal area represented mucoepidermoid carcinoma. In the lip two thirds of all tumors were benign, malignant mixed tumor and adenocarcinoma represented the majority of the remaining third of labial malignant tumors.

The symptomatology and signs in these patients depend on the location of the tumor. The lesions of the oral cavity appear as a painless mass. Patients with intranasal and nasopharyngeal tumors have epistaxis, nasal obstruction, pain, and nasal discharge. Hypopharyngeal and laryngeal tumors manifest themselves through symptoms of dysphagia, "sore throat," and hoarseness. Occasionally the malignant tumors become manifest as metastasis in a cervical node or less commonly at distant sites.

The types of minor salivary gland tumors are classified as adenoid cystic carcinoma, benign mixed tumor, malignant mixed tumor, mucoepidermoid carcinoma, and adenocarcinoma (1, 6, 58). The prognosis of the malignant tumors of the minor salivary glands is poorer than with the malignant tumors of the major salivary glands. Surgical resection is the treatment of choice for the untreated malignant tumors and for resectable local recurrences. Radiotherapy has been used preoperatively for local recurrences not amenable to excision, and for palliation. It has also been used postoperatively after incomplete removal of the tumor.

Case 10. A Negro man, age 52 years, was seen in the ENT clinic complaining of right nasal obstruction which had been present for about three months. The onset was insidious and non-relenting. There was a small amount of blood-tinged mucus, but no pain, hearing loss, nor history of trauma. The past history was unremarkable.

Examination showed a lobulated, red mass (1 2 cm) in the right choana which originated from the floor of the nose and extended onto the right inferior turbinate. Serous otitis was not present. A transnasal biopsy demonstrated adenocystic carcinoma. Sinus x ray films and contrast studies of the sinuses excluded their involvement. The tumor mass was removed from the nose through right lateral rhinotomy. It was found to be attached to the floor of the nose and the posterior border of the inferior and middle turbinates. The patient is free of recurrence three years post op.

The tumor was described as being a moderately cellular glandular tumor presenting a distinct cribriform pattern. There were cords and nests of small cells with hyperchromatic nuclei. Mitotic activity was not seen.

Case 11. A white man, age 64 years, complained of a "scratchy feeling" in the left side of his throat for some six months. Initial examination showed a small, whitish, round (2 × 3 mm) growth on the ventricular fold anterior to the left arytenoid cartilage. The cartilage moved well, the vocal cord was normal, and the voice clear. The patient had had a spontaneous pneumothorax some five years earlier supposedly from emphysema. He had smoked excessively for many years.

A biopsy of the lesion was reported as a mixed tumor of a minor salivary gland, but it was not certain whether it was benign or malignant. Under general anesthesia, the larynx was opened through laryngofissure, the entire left ventricular fold and the suprastructure of the left arytenoid cartilage were removed while preserving the vocal process and cord.

Although the tumor was adequately excised, considerable discussion regarding the pathological diagnosis ensued. The slides were sent to AFIP and the final consensus of opinion established a malignant mixed tumor of a minor salivary gland. The patient has been free of disease for four years.

Case 9 A white man age 63 years, was admitted to the ENT service with a 2 1/2 year history of bilateral submandibular swelling. These masses began as small nodules and gradually increased in size There was no history of pain, bleeding, or abnormal saliva tion. Weight loss of 18 pounds occurred over the past two years. The remainder of the past history was unremarkable.

Examination showed bilateral submandibular masses. Each was about 4 × 5 cm in size and was slightly lobulated with cystic areas. There was no cervical lymphadenopathy Chest x ray films demonstrated a moderate amount of pulmonary fibrosis without evidence of metastatic disease Sinus x ray films were normal. No primary lesion could be detected, nor was there evidence of metastatic disease. After a biopsy had indicated mucoepidermoid carcinoma, both submandibular masses were widely excised.

Several months later the patient developed posterior and anterior cervical adenopathy Lymph node biopsy was reported as carcinoma. The results of further examinations of nasopharynx, oropharynx and sinuses were normal. The patient then received 5000 R cobalt with good initial response. However two months later local recurrence occurred at the angle of the left mandible and multiple metastatic nodules were scattered throughout both lung fields. The patient died from his disease 15 months after excision of the tumors.

Malignant Tumors of the Minor Salivary Glands

Our first series of rare tumors (47) reported three tumors of the minor salivary glands, two benign mixed tumors of the soft palate, and one adenocarcinoma of the hard palate. This present series includes three patients one with adenoid cystic carcinoma of the nasopharynx, another with a malignant mixed tumor of the ventricular fold and a mixed tumor of the nasal septum. The latter tumor was discussed above in the section of Tumors of the Nasal Septum

Tumors of the minor salivary glands occur with significant frequency in the upper respiratory tract. They are morphologically similar to those that arise in the major salivary glands (28) The tumor usually arises from accessory salivary glands or from mucous glands (1) These tumors, both benign and malignant occur predominantly in males, mostly in patients 40 to 60 years of age The most common tumor type and the most frequent location vary among reports. Common sites are the palate nasopharynx, alveolus, tongue sinuses, nasal septum oral mucosa tonsil, pharynx (oro- and hypo-) and the larynx. The larynx is an uncommon location for this rare tumor Sabri and Hajjar (57) reported a case of malignant mixed tumor of the vocal cord and found 37 cases reported in the world literature.

Conley (17) found that tumors of the minor salivary glands were about five times less common than parotid tumors which constitute approximately 88% of all major salivary gland tumors. He stated that 56% of the minor salivary gland tumors were malignant. Moreover 55% of all minor salivary gland tumors were found in females and they were most common in the fifth decade of life. More than half of the tumors occurred in the palate and 58% of these were malignant In the group of tumors of the tongue floor of the mouth and alveolus, 95% were malignant The percentage of malignancy for the buccal area, lip, and nasal cavity was 65% 35% and 50% respectively The predominant carcinoma in the palate and the nasal cavity was adenoid cystic carcinoma while the majority of tu-

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Adenocarcinoma of the Tongue

Malignant tumors of the tongue are quite frequent. The incidence varies with geographic location from 1% of all cancer deaths in the United States to 14% as reported from India (54). About 95% of all lingual cancers are of squamous cell or epidermoid type (23, 39). The other 5% consist of rhabdomyosarcomas and tumors of minor salivary glands. When Gobbel et al. (31) reported 100 cases of tongue cancer, 109 of these were squamous cell carcinomas and only one was a rhabdomyosarcoma. Owen (50) found that the adeno-cell type constituted less than 2% of all carcinomas of the tongue. Burbank et al. (12) reported on 43 glandular tumors of the tongue observed among more than 3 800 cases of benign and malignant lesions of the tongue. Twenty two (51%) of these 43 tumors were cylindromas which are the most common glandular tumors, eleven (26%) were of mucoepidermoid type, nine (21%) were adenocarcinomas, the remaining glandular lesion was a mixed tumor. Harrold (32) reported 281 patients with cancer of the base of the tongue and 7% of these showed adenocarcinoma.

Watson (68), in his series of 41 intra-oral adenocarcinomas, found that 17% of the tumors involved the base of the tongue. Burbank et al. (12) noted that some of these adenocarcinomas strongly resembled thyroid adenocarcinoma. An origin from lingual thyroid tissue suggests an attractive but unproven hypothesis, especially of those tumors at the base of the tongue.

Good results can be obtained with radical surgery of the glandular tumors. The results depend on the anaplasticity of the tumors, their size and the spread. Five of the nine patients with adenocarcinoma reported by Burbank et al. (12) died of their disease seven months to seven years after treatment. The four who lived were seen 3 to 16 years after treatment.

Case 12. A white man, age 83 years, noticed a small raised lesion on the right side of his tongue 1 $^1/_2$ to 2 years before seeking medical advice. During the recent weeks prior to treatment, this lesion became ulcerated and was mildly uncomfortable. The patient had been a pipe smoker for many years, his daughter had died of tongue cancer and he had a weight loss of 17 pounds in two months.

Examination showed a large, ulcerated, indurated lesion on the right margin of the tongue, extending to the border of its anterior one third and posteriorly to the circumvallate line. It extended downward to the floor of the mouth but not to the mandible and deep inside the tongue. There was a large fixed node in the right submandibular area. The man also had a small hemangioma on the right lower lip, another on the left upper lip, and still a third on the right base of the tongue. A biopsy of the mass showed adenocarcinoma. It was classified as $T_3N_1M_0$, Stage III.

The patient was advised to have preoperative radiotherapy followed by a hemiglossectomy, right partial mandibulectomy and right radical neck dissection. He refused this plan and was treated with external radiotherapy resulting in an excellent regression of the primary lesion. However, cervical nodes did reappear, a radical neck dissection was performed, and radium needles were placed into the tongue. Fifteen of the 44 cervical lymph nodes contained metastatic tumor. The patient was free of tumor for two years, but later he could no longer be traced by mail.

Adenoid Cystic Carcinoma of the External Auditory Canal

The external auditory canal is lined with skin that is continuous with the skin of the auricle. The skin covering the cartilaginous portion of the meatus contains hair follicles, sebaceous glands, and modified sweat glands or ceruminal glands. It is from these structures that glandular tumors of the external auditory canal originate.

The exact incidence of cancer of the external auditory canal is uncertain for several reasons. Case reports are isolated and not always well documented. Some larger series fail to separate external auditory canal lesions from middle ear and mastoid lesions. Broders (11) estimated that 84% of aural cancer involved the auricle, slightly more than 14% the auditory canal, and less than 2% the middle ear and mastoid. There is no sex predominance in carcinoma of the external auditory canal (35). Most of the tumors occur between the ages of 45 and 65.

The malignant tumors of external auditory canal can be divided into epithelial tumors and mesenchymal tumors (35). The epithelial tumors include squamous cell carcinoma, basal cell carcinoma, and malignant melanoma. The glandular tumors arise from the glands mentioned above and are classified as adenocarcinoma, also called adenoid cystic carcinoma, cylindroma, adenomyoepithelioma, and basiloma (60). Ash et al. (1) consider the ceruminoma to be a benign tumor stating that they had no record of an authentic ceruminal gland tumor that had metastasized. Batsakis et al. (7) considered one of their two reported cases of "ceruminomas" to be malignant on the basis of local aggressiveness and infiltration. O'Neal and Parker (49) found ceruminous gland tumors to carry a poor prognosis.

The treatment of malignant tumors of the external auditory canal is excision with adequate margins of those tumors that are limited to the external canal and do not invade the cartilaginous or bony capsule. Unfortunately many cases represent advanced tumors with extension into the middle ear and temporal bone. A mastoidectomy in combination with removal of the external auditory canal sometimes adequately removes the tumor. Parsons and Lewis (53) advocated a subtotal resection of the temporal bone after Campbell et al. (13) had originally described a total resection for more extensive neoplasms. Radiation has been used preoperatively, postoperatively, as the sole means of treatment, and for palliation.

We had two patients with a malignancy of the external canal, one with an adenocystic carcinoma and the other with a ceruminoma. The ceruminoma is exceedingly rare and will be published in detail by Louis and Smith (36).

Case 13. A Negro man, age 48 years, complained of intermittent throbbing pain in the left ear of one year's duration. There had been a rapid deterioration of hearing in that ear during the preceding three months as well as an intermittent bloody discharge. His local physician biopsied a lesion in the external auditory canal which was diagnosed as adenocystic carcinoma. He was referred to the ENT service for further management. The remaining past history was unremarkable.

Examination was normal except for the findings in the left ear. There was frequent bleeding within the ear canal. The left tympanic membrane was eroded by a grayish papillary growth extruding from the middle ear. There was no evidence of purulence or adenopathy. Mastoid x-ray films demonstrated clouding on the left with loss of the cellular structure (Figs. 10–11). The skull x-ray films were normal.

The patient underwent a left radical mas-

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Watson (68), in his series of 41 intra-oral adenocarcinomas, found that 17% of the tumors involved the base of the tongue. Burbank et al. (12) noted that some of these adenocarcinomas strongly resembled thyroid adenocarcinoma. An origin from lingual thyroid tissue suggests an attractive but unproven hypothesis, especially of those tumors at the base of the tongue.

Good results can be obtained with radical surgery of the glandular tumors. The results depend on the anaplasticity of the tumors, their size, and the spread. Five of the nine patients with aden carcinoma reported by Burbank et al. (12) died of their disease seven months to seven years after treatment. The four who lived were seen 3 to 16 years after treatment.

Case 12. A white man, age 83 years, noticed a small raised lesion on the right side of his tongue $1\,^1/_2$ to 2 years before seeking medical advice. During the recent weeks prior to treatment, this lesion became ulcerated and was mildly uncomfortable. The patient had been a pipe smoker for many years, his daughter had died of tongue cancer, and he had a weight loss of 17 pounds in two months.

Examination showed a large, ulcerated, indurated lesion on the right margin of the tongue extending to the border of its anterior one third and posteriorly to the circumvallate line. It extended downward to the floor of the mouth but not to the mandible and deep inside the tongue. There was a large fixed node in the right submandibular area. The man also had a small hemangioma on the right lower lip, another on the left upper lip and still a third on the right base of the tongue. A biopsy of the mass showed adenocarcinoma. It was classified as $T_2N_1M_0$, Stage III.

The patient was advised to have preoperative radiotherapy followed by a hemiglossectomy, right partial mandibulectomy and right radical neck dissection. He refused this plan and was treated with external radiotherapy resulting in an excellent regression of the primary lesion. However, cervical nodes did reappear, a radical neck dissection was performed, and radium needles were placed into the tongue. Fifteen of the 44 cervical lymph nodes contained metastatic tumor. The patient was free of tumor for two years, but later he could no longer be traced by mail.

tion. She did not have vertigo, ear drainage, or history of trauma to her head or ear. There was no family history of hearing loss.

Examination revealed a granulomatous tissue filling the right external ear canal. There was partial paralysis (peripheral type) of the fifth and seventh nerves and a conductive hearing loss, all on the right side. Mastoid X-ray films demonstrated clouding of the mastoid air cells with destruction of some of the bony septa. The patient was thought to have chronic otitis media with cholesteatoma.

Radical mastoidectomy was performed during which a frozen section of the tissue was reported as a neoplasm. Permanent sections of the tumor were diagnosed as ceruminoma. Tomograms of the right temporal bone showed involvement of the petrous apex. A partial abducens nerve paralysis developed on the right side two weeks after surgery and disappeared three months later believed to be due to petrositis. The patient was treated with radiotherapy postoperatively. Seven months later a recurrence of the ceruminoma appeared in the right nasopharynx and tomographic films showed advanced destruction around the apex of the temporal bone.

Malignant Melanoma of the Hard Palate

Malignant melanoma was discussed at the beginning of this paper in connection with the nasal septum. This unpredictable tumor constitutes about 1.5% of human cancers and approximately 20% of all melanomas occur in the region of the head and neck (18). Melanoma of the oral cavity is very rare. Most of the melanomas of the mucous membrane occur in the nose and sinuses. Only one melanoma of the hard palate was discovered in the M. D. Anderson Hospital series (2). Simons (59) reported 257 patients with malignant melanoma of the head and neck seen at the Mayo Clinic from 1950 through 1967. He had only two patients in whom the melanoma arose from the intraoral mucous membrane, but the exact site was not mentioned.

Chaudhry et al. (16) in a detailed review of 105 cases of malignant melanoma of the oral cavity found 38 cases involving the hard palate. They found oral cavity melanomas to occur twice as often in males. The average age was 50.5 years and a marked tendency for both regional as well as distant metastasis was noted. The prognosis for oral mucosal melanomas is much more serious than that of cutaneous melanomas.

Moore and Martin (46) reported 1 546 patients with malignant melanoma seen at the Memorial Hospital Center from 1930 through 1948. Among these, 492 (27.6%) involved the head and neck region, but only 27 (1.7%) originated in the oral and upper respiratory tract. Three of these lesions were located in the hard palate.

Case 15. A Negro man, age 62 years, was seen at the Medical Center for a neck mass which had been present for about three months. A biopsy of a pigmented, ulcerative



Fig. 12. Malignant melanoma of the hard palate, metastatic to the lymph nodes of the anterior triangle of the left neck (Case 15).



Fig. 10. Law projection of both mastoids shows a loss of the cellular structure and clouding of the left mastoid (Case 13).

toidectomy and the tumor was incompletely resected because of extension into the labyrinth and cochlea. The patient received 5000 R cobalt to the left temporal area.

The patient was readmitted one year later because of the development of hoarseness and extensive left cervical adenopathy. X-ray films showed progressive destruction in the region of the left mastoid and petrous bone. The left vocal cord was paralyzed in the paramedian position and did not move for phonation. The left neck mass proved to be metastatic adenoid cystic carcinoma. The man eventually developed massive recurrence of the tumor and died 23 months after diagnosis.

Case 14. A Negro woman, age 50 years, complained of a gradual hearing loss, tinnitus, and right sided headaches of three years dura-



Fig. 11. The right mastoid is normal (Case 13).

among the male (11 9), again the Negro (12: 8), but in the older age groups (14 6).

There have been few reports of bilateral salivary gland tumors. Bilateral papillary cystadenoma lymphomatosum (Warthin's tumor) is well known. As stated before, Frazell (29) saw 21 patients with multiple salivary gland tumors. Sixteen of these had bilateral Warthin's tumors in the parotid gland. One patient had an acinic carcinoma of the parotid and benign mixed tumor of the submaxillary glands. The simultaneous, bilateral occurrence of a mucoepidermoid carcinoma of both submaxillary glands in our case 9 is certainly rare as far as we could ascertain this is the first report of such a case.

Summary

This is the third of a series of rare tumors of the head and neck. The first series contained nine benign and five malignant tumors of rare type. The second series presented 15 benign tumors. The 15 cases reported in this paper are all rare malignant tumors. They are discussed as to their incidence location, diagnosis, treatment, and prognosis. A comparison of the benign and malignant tumors of these three series suggests two different trends of incidence in these rare lesions. The simultaneous, bilateral occurrence of a mucoepidermoid carcinoma of both submaxillary glands is described for what appears to be the first time.

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ion on the hard palate (Fig. 12) indicated lignant melanoma. The patient had had no ight loss.

Examination showed a mass (3 × 3 cm) in left neck over the carotid bifurcation ere was a black, ulcerated lesion (2 × 3 cm) r the hard palate He also had a papillatous lesion which filled the right nasal ity arising from the right middle turbinate. us x ray films showed massive clouding of right antrum and ethmoids. Liver scan g bone survey and brain scan were nor l. The chest x ray films were normal at t time. Biopsy of the nasal lesion showed enign squamous papilloma. Exploration of the right maxillary sinus through a Caldwell-Luc approach explained its mucosal proliferation as the result of chronic sinusitis. The palatal lesion was removed by the Plastic Surgery Service by wide excision of the hard palate and the entire alveolar ridge of the maxilla. At the same time we removed the medial wall of the right antrum with the attached papilloma. A left radical neck dissection was also done. A temporary maxillary prosthesis was inserted at the time of surgery

Subsequently the patient developed a pulmonary mass. He was alive with metastasis six months postoperatively at the time of this writing.

Discussion

ce its organization in 1963 the Division Otolaryngology of the Department of Sur y at the University of Mississippi Medical iter has admitted almost 10 000 patients the ENT clinic During these seven years, rare benign and malignant tumors of head neck have been seen and reported in a es of three papers. The first series included rare tumors, both benign and malignant. second series recorded 15 rare benign tu rs. This third series presents 15 rare ma ant tumors of the head and neck.

t was pointed out in the first two series of tumors that there was an unusually high portion of the cases occurring in the Negro (26 of 29) This could not be explained. was suggested to be of genetic influence present series of malignant tumors re ts an almost equal distribution in the icasian and Negro races. As expected the ignant tumors occurred in an older age up when compared with the benign tu rs.

he pathologic classification of these 15 tumors is as follows one squamous cell cinoma (septum) two malignant melano (septum and hard palate) two malignant mixed tumors (septum and submaxillary gland) one angiosarcoma of the maxillary and ethmoid sinuses two adenoid cystic carcinomas (nasopharynx and ear canal) one adenocarcinoma (parotid gland) one carcinoma of a minor salivary gland (ventricular fold in the larynx) one bilateral simultaneous, mucoepidermoid carcinoma of submaxillary glands, one rhabdomyosarcoma of the ethmoids, antrum and orbit one mucoepidermoid carcinoma of the parotid gland one ceruminoma of the ear canal and one papillary adenocarcinoma of the tongue Nine tumors occurred in males and six in females. Seven occurred in the Caucasian race and eight in the Negro race The average age was nearly 49 years.

When the three series are combined, as in Table 3 the trends encountered in the discussions of the two first series seem to emerge again albeit with a further modification. The three series are compared best by examining the benign and malignant lesions separately The following trends are seen to emerge the 24 benign rare tumors seem to predominate among the female (15 9), the Negro (22 2) and the younger age groups (17 7) The 20 malignant tumors seem to predominate

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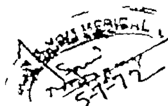
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Introduction

Although there is considerable information about how the human body compensates for cold exposure and much has been written regarding the thermal homeostasis of man, very little is known about the effects of cold air temperature on the human auditory system. Carco (1952) summarized the existing literature in this area. The type of hearing loss he reported on—cases where rapid, sudden, and permanent deafness (unilateral or bilateral) occurred following cold exposures of varying durations and severity—was referred to as a cochlear block. The problem was sometimes noted to be accompanied by tinnitus of low pitch and or vestibular disturbances. Carco offered a variety of theories as to the physiology surrounding these observed severe sensorineural hearing losses as well as an interesting hypothesis to explain the selectivity of the effect of cold air exposure on hearing. He suggested that this may be based on a pre disposition determined by a variety of physical factors

Based on studies with laboratory animals there is also some evidence to suggest that extreme cold does have an effect on the cochlea and on hearing. This has been demonstrated both by altering body temperature and by directly changing the temperature in the cochlea. Kahana, Rosenblith, and Galambos (1950), using hamsters, and Gulick and Cutt (1960) using guinea pigs, demonstrated that the magnitude of the cochlear response was reduced when body temperature was reduced. Patterson et al (1968), using turtles, and Chambers and Loccina (1956), with a cat, obtained similar results when they locally reduced cochlear temperature without affecting the animals overall body temperature.

When specific human muscle tissue is exposed to cold, its activity is demonstrably diminished due to the generalized stiffness of the muscle (which is associated with the constriction of arteries, veins, and capillaries and the resultant general reduction of blood supply to the muscles involved). The tensor tympani and stapedius muscles would presumably respond similarly to chilling, the end result being that neither the tympanic membrane nor the auditory ossicles would move as freely as in the normal state. In addition, it is possible that the tympanic membrane might stiffen as a direct reaction to becoming chilled (reduced blood supply etc.). Also, the cold air may reach and affect the action of the tympanic membrane directly (via the external auditory meatus) and/or the middle ear muscles indirectly (through the venal blood supply and the mucosal lining of the nasopharynx).

It should be noted that head temperature (measured at the back of the nasal cavity and the interior of the ear) may not correlate well with body core temperature. Benzinger (1963) believed that this was possibly due to the passing of cooler blood returning from the ear lobe and surrounding area. The cooled blood in the descending veins would be adjacent to some of the ascending arteries which supply the tympanic membrane.

This resulting increased stiffness might be expected to affect the impedance characteristics of the middle ear along with the air conduction hearing thresholds. Based on the

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This resulting increased stiffness might be expected to affect the impedance characteristics of the middle ear along with the air conduction hearing thresholds. Based on the

research reviewed one could conclude that bone-conduction thresholds might also be affected if the cold air reached the cochlea. If the persons involved were warmly dressed as they normally would be when out of doors in cold weather the cold could only be passed to the inner ear through the middle ear (local cooling) since overall body core temperature would not be affected.

Empirical observations made earlier in our laboratory suggested that when normal hearing human subjects spent approximately 15 min out of doors in cold weather immediately prior to pure tone audiometric testing a conductive type stiffness tilt was observed instead of the expected normal audiogram. In most audiology clinics audiometric testing begins very shortly after the client arrives at the testing suite It is not unusual for clients who are warmly dressed but with their heads exposed to spend ten or fifteen minutes out of doors in temperatures below 0 F

Purposes of the Study

The primary purpose of this study was to determine the effects of extremely cold air temperatures on the human peripheral auditory system Assuming cold exposure takes place immediately before a person is tested, the specific questions asked were What effects will this exposure have on pure-tone thresholds, middle ear impedance and tympanic temperature?

A second function was to check the reliability of the test results obtained following the first 20 min exposure The third purpose was to determine the critical length of exposure (to −7 F) necessary before air-conduction thresholds are affected. The final purpose was to examine the recovery from the effects found.

Experiment I
Effects of the Initial Twenty Minute Exposure

METHOD

Forty-six college students with normal hearing and good general health were selected to serve as subjects. They were chosen on the basis of age (between 17 and 26 years) sex (approximately an equal number of males and females) and body build (only subjects judged to be of approximately average height and weight were used) In addition each subject's ears were inspected by an otologist to determine that the tympanic membranes were intact and healthy and to rule out any subjects whose external auditory canals were not of normal size, configuration and angle of inclination. Lastly whenever necessary the ears were cleaned of wax and/or other material

Pre- and post-exposure pure tone air and bone-conduction thresholds, acoustic impedance readings and tympanic temperature measurements were made in an empty and quiet but not sound-treated room located in the Michigan State University Food Stores building. Ambient noise levels in this room were 65 dB (C Scale) and 43 dB (A Scale) as measured with a sound level meter (Bruel and Kjær type 1613) employing a one inch sound field condenser microphone (Bruel and

Kjær type 4131). The noise levels were felt to be sufficiently low so as not to seriously interfere with pure-tone air-conduction threshold measurement (Cox, 1955). However it was necessary to use ear muffs (American Optical Company Model 1200) in order to obtain bone-conduction threshold measurements from the normal listeners.

For the cold exposures the subjects were placed in a large (120 feet by 120 feet) commercial type freezer located across a hallway from the testing room. The temperature in the freezer ranged from −3 F to −10 F during the course of the investigation and averaged −7.7 F. The doors to the freezer can be electrically or manually operated both from within and from without.

The subjects were instructed to eat nothing following lunch on the day they were to participate in the research. Immediately upon their arrival they were provided with a donut and a glass of milk and then waited 30 min to one hour before testing began. In this way an attempt was made to rule out differences in body metabolism as a complicating factor.

Each of the initial group of 46 subjects had their pure-tone air-conduction thresholds determined in both ears at octave intervals from 125 Hz through 8000 Hz using a portable audiometer (Beltone, Model 12 C) with TDH 39 10z earphones mounted in MX 41/AR cushions. This audiometer was calibrated to ISO 1964 standards. Attenuator linearity permitted testing in 2 dB steps. A 20 dB attenuation pad was inserted in one air-conduction line and in the bone-conduction circuit. American Optical Company ear muffs were used in measuring occluded bone-conduction thresholds. The bone-conduction oscillator used was a Radioear B 70 A white dot.

The pure-tone air-conduction system was calibrated weekly during the course of the investigation using an artificial ear assembly (Bruel and Kjær type 4152) employing a condenser microphone (Bruel and Kjær type 4132) with the associated sound level meter (Bruel and Kjær type 2203) and octave band filter network (Bruel and Kjær type 1613). The bone-conduction system was calibrated at the beginning of the research and again at its conclusion in the standard manner using an artificial mastoid (Beltone Model M5A) and was found to be satisfactory.

Because of the high ambient noise level in the test environment, conditions were not ideal for bone-conduction testing. It was desireable, nevertheless, to obtain the most sensitive and reliable bone-conduction thresholds possible under these conditions. Therefore, bone-conduction thresholds were measured without masking at octave intervals from 250 Hz through 4000 Hz using a forehead oscillator placement and with the ears occluded. Occlusion of the ears in normal subjects results in enhanced bone-conduction sensitivity particularly in the low frequencies (Naunton, 1957). Masking was not used in order to avoid the central masking phenomenon which produces an adverse effect on sensitivity of about 5 dB (Dirks, 1964). It was assumed that the bone-conduction responses were consistently elicited from the better ear (since masking was not used) and that, since both ears were exposed to the cold, they would both be affected in the same way. Forehead oscillator placement was used because it has been demonstrated to yield more reliable thresholds than mastoid placement (Dirks, 1964).

Pure-tone testing was carried out employing the Hughson–Westlake ascending technique as described by Carhart and Jerger (1959) with the exception that thresholds were measured in two decibel steps.

Following the first air-conduction threshold testing, the better ear was identified as the test ear. Air-conduction thresholds were obtained a second time in this ear. After a 20 min break, air-conduction thresholds were obtained a third time in the test ear. The bone conduction threshold testing was also done at this time. This provided pre-exposure thresholds and eliminated the complication of having thresholds improve with practice and

research reviewed, one could conclude that bone-conduction thresholds might also be affected if the cold air reached the cochlea. If the persons involved were warmly dressed, as they normally would be when out of doors in cold weather the cold could only be passed to the inner ear through the middle ear (local cooling) since overall body core temperature would not be affected

Empirical observations made earlier in our laboratory suggested that when normal hearing human subjects spent approximately 15 min out of doors in cold weather immediately prior to pure tone audiometric testing a conductive type stiffness tilt was observed instead of the expected normal audiogram. In most audiology clinics, audiometric testing begins very shortly after the client arrives at the testing suite. It is not unusual for clients who are warmly dressed but with their heads exposed to spend ten or fifteen minutes out of doors in temperatures below 0 F

Purposes of the Study

The primary purpose of this study was to determine the effects of extremely cold air temperatures on the human peripheral auditory system. Assuming cold exposure takes place immediately before a person is tested the specific questions asked were What effects will this exposure have on pure tone thresholds, middle ear impedance and tympanic temperature?

A second function was to check the reliability of the test results obtained following the first 20 min exposure The third purpose was to determine the critical length of exposure (to −7 F) necessary before air-conduction thresholds are affected. The final purpose was to examine the recovery from the effects found.

Experiment I
Effects of the Initial Twenty Minute Exposure

METHOD

Forty six college students with normal hearing and good general health were selected to serve as subjects. They were chosen on the basis of age (between 17 and 26 years) sex (approximately an equal number of males and females) and body build (only subjects judged to be of approximately average height and weight were used) In addition each subject's ears were inspected by an otologist to determine that the tympanic membranes were intact and healthy and to rule out any subjects whose external auditory canals were not of normal size, configuration and angle of inclination. Lastly whenever necessary the ears were cleaned of wax and/or other material.

Pre- and post-exposure pure tone air and bone-conduction thresholds, acoustic impedance readings and tympanic temperature measurements were made in an empty and quiet but not sound-treated room located in the Michigan State University Food Stores building Ambient noise levels in this room were 65 dB (C Scale) and 43 dB (A Scale) as measured with a sound level meter (Bruel and Kjær type 1613) employing a one inch sound field condenser microphone (Bruel and

Acoustic impedance in the plane of the ear drum is based upon the following formula.

$$Z_e = \frac{Z_1 \times Z_2}{Z - Z_2}$$

where Z is the point of minimum compliance of the middle ear system and approximately represents the actual canal volume and Z_2 is the point of maximum compliance of the middle ear system.

A measurement of middle ear pressure was noted for 11 subjects and, in five cases, the intra-aural stapedius muscle reflex threshold to a 250 Hz stimulus was measured. (This frequency was chosen because it was within the range of frequencies affected by the cold exposure.) Compliance readings at a variety of pressure settings were taken for 15 subjects in order to determine the compliance of the ear drum under varying canal pressure conditions. The headset was not removed and sensitivity and pump controls were returned to zero. The pressure in the canal was then raised to + 200 mm and the sensitivity control set to position 1. The compliance control was adjusted so that the balance meter reading was 10. The canal pressure was then reduced in 40 mm steps and the balance meter readings noted at each of the pressure settings tested. + 160, + 120, + 80, + 40, 0, − 40, − 80, − 120, − 160, and − 200 mm water pressure.

Subjects were allowed to dress as warmly as desired (leaving the head and ears exposed) before entering the freezer for 20 min. They were instructed not to run, jog, exercise, or engage in any type of strenuous activity.

The order of tests was: air-conduction thresholds, bone-conduction thresholds, impedance (when measured), and temperature (when measured). The same test order was followed during the post-exposure session. When hearing thresholds shifted, the subjects were not allowed to lea e the building until the affected thresholds had returned to pre-exposure levels. Generally a threshold check was made one hour following the conclusion of the cold exposure. If thresholds had not then returned to pre-exposure levels, another check was made 15 or 20 min later. In all cases thresholds had returned to normal within 80 min following the conclusion of the cold exposure.

RESULTS

Table I shows the means and standard deviations for the shifts in occluded bone-conduction thresholds and the significance levels of the mean shifts (from pre-exposure to post exposure readings) for 46 subjects following a 20 min cold exposure. The primary statistic shown throughout the Results section is a one-way analysis of variance.

The mean shifts found by occluded bone-conduction testing following the cold exposure were extremely small (the largest mean shift being less than 1 dB) and none of the shifts differed significantly from zero ($P \geq 17$). In addition, no subject showed consistent bone-conduction threshold shifts following the cold exposure. It would appear then, that 20 min of cold exposure under these conditions does not measurably affect bone-conduction thresholds.

The means and standard deviations of the pre and postexposure air-conduction thresholds and the shifts in air-conduction thresholds plus the significance levels of the mean shifts for the total group of 46 subjects are shown in Tables II and III.

Table I. *Means, standard deviations and significance levels of mean shifts in occluded bone-conduction thresholds found for the 46 subjects following a 20 min cold exposure*

	Frequency (Hz)				
	250	500	1000	2000	4000
Mean[a]	−0.59	0.44	0.15	−0.29	0.83
Standard deviation[a]	7.43	6.09	4.13	5.72	3.82
Significance level of mean shift	0.62	0.65	0.82	0.75	0.17

[a] In dB re pre-exposure level.

thereby possibly contaminate the results obtained following the cold exposure

For 37 of these subjects tympanic temperature was also determined prior to the cold exposure using a tympanic thermometer (Radiations Systems, Inc. Model no. 104382). This thermometer is battery operated. It is a small (13 × 9 × 7") portable (14 1/3 lbs) cabinet from which a six foot lead connects to interchangeable disposable probes or thermocouples which consist of constantan and copper wire embedded in thin polyethylene tubing The end of the wire is looped and the soldered junction is also embedded in the tubing. Thermocouple wires surrounded with polyethylene tubing facing the interior of the auditory canal with their free ends help to hold the probe in place against the tympanic membrane while the temperature measurement is being made. The use of a thermoelectric method (as opposed to thermistor measurements) requires amplification This is provided so that readings may be made to 1/100 C. Using this equipment tympanic temperature may be obtained within 15 sec at an accuracy of up to 0.2 C.

The thermocouple wires were inserted into the external canal until they reached the tympanic membrane. This point was determined in three ways. Tactually when the probe reached the drum membrane there was the distinct feeling of a sudden resistance to further insertion Secondly the subject usually reported a slight twinge when the probe touched the tympanic membrane Thirdly the highest temperature reading possible in the canal is on the drum membrane. Therefore a final check to correct placement was to slightly withdraw the probe If the probe was correctly placed originally the tympanic temperature dropped somewhat when the probe was withdrawn slightly Then it was replaced.

For some subjects a variety of impedance measurements were made using a Madsen Acoustic Bridge Model ZO 70. This bridge is composed of a pure-tone generator which produces a probe tone of 220 Hz, a pressure system composed of a pump and an electromanometer and a section for acoustic impedance measurements. The pure-tone generator is connected to an earphone and a tube from the earphone conveys the 220 Hz tone to the probe unit (which is part of the headset) and then into the external auditory meatus. The meatus is sealed forming an air tight cavity Some of the acoustic energy is absorbed the remainder is reflected by the tympanic membrane and conveyed back to a microphone in the probe unit through a second tube. A third tube is connected to the probe unit from the pressure system which allows positive or negative air pressures to be applied to the external auditory canal. The sound pressure level in the canal may be varied (Madsen manual).

When the compliance control is adjusted to give a zero reading on the balance meter the sound pressure level in the canal is precisely 95 dB (When the microphone voltage equals 18 volts the voltage at the balance meter is zero since it has been perfectly balanced with the DC 18 volt supply provided by the bridge on the other side of the meter) With this equipment the reactance or compliance of the ear may be read on a scale in acoustic ohms or in cc and the impedance calculated in acoustic ohms. In addition it is possible to determine the presence or absence of the middle ear muscle reflexes, measure the compliance of the ear under a variety of air pressures and perform other impedance measurements.

The headset was fitted with a TDH 39 earphone and an eartip of suitable size was selected and fitted onto the probe The probe was inserted into the meatus and then checked to ascertain that an airtight seal had been achieved (by increasing the canal pressure to +200 mm water pressure and waiting a few seconds to observe on the pump control that the pressure had held steady). Measurement of acoustic impedance in the plane of the ear drum was made in 45 subjects according to the directions given in the Madsen manual

shifted downward by the cold exposure. Fig. 1 displays the pre- and post-exposure air-conduction thresholds for the shifter group. Fig. 2 presents the same data for the remaining 26 subjects classified as non-shifters. The threshold shifts found in the shifter group were greatest in the low frequencies (in the direction of poorer hearing), disappeared in the mid frequencies and became very slightly improved in the high frequencies. The non-shifters, on the other hand, showed virtually no threshold shifts at any frequency and the variation around zero appeared to have no pattern. The differences in the air-conduction shifts between the two groups were significant at 125 Hz, 250 Hz, and 500 Hz (at a level of 0.0005), whereas none of the pre-exposure thresholds were significantly different from those found for the total group. When the threshold shifts were correlated with pre-exposure thresholds at 125 Hz and 250 Hz, they were significantly related and there was a marginal correlation at 500 Hz. In all three cases the relationship was negative indicating that the better the original threshold at these frequencies, the greater the size of the threshold shift.

The ranges, means, and standard deviations

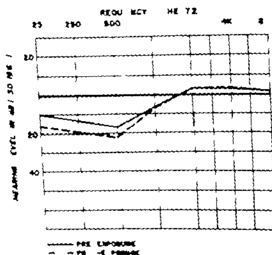


Fig. 1. Audiogram showing the mean pre- and post-exposure air-conduction thresholds for the shifters (N [illegible])

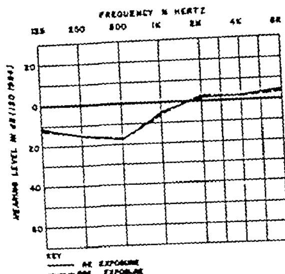


Fig. 2. Audiogram showing the mean pre- and post-exposure air-conduction thresholds for the non-shifters (N = 26).

of the pre- and post-exposure tympanic temperature readings and shifts in tympanic temperature as well as the significance level of the mean shift is given in Table IV. The mean pre-exposure temperature was 98.00 F which agrees well with the figure reported by Ganong (1967) (98.10 F) for healthy young adults. The mean post-exposure temperature reading was 97.28 F showing a mean shift of −0.72 F. In no case did tympanic temperature rise following the cold exposure although in a few cases it was unchanged. There was a slight difference in the pre-exposure temperatures of the shifters and non-shifters.

Table IV. *Ranges, means and standard deviations of the pre- and post-exposure tympanic temperature readings and shifts in tympanic temperature for the total group of 37 subjects*

	Range (°F)			
	Minimum value	Maximum value	Mean (°F)	S.D. (°F)
Pre-exposure	96.70	99.50	98.00	0.35
Post-exposure	95.40	98.40	97.28	0.68
Temperature shift	2.40	0.00	−0.72*	0.55

* Significant at less than the 0.0005 level.

Table II *Means and standard deviations of the pre- and post-exposure air-conduction thresholds found for the 46 subjects*

	Frequency (Hz)						
	125	250	500	1 000	000	4 000	8 000
Pre-exposure A/C thresholds							
Mean	10.80	15 13	17.59	5.91	−1 98	−2.33	[illegible].67
S.D	5.28	4.50	3.49	3.99	6.69	5.31	8.71
Post-exposure A/C thresholds							
Mean[a]	13.97	17.65	19 42	6.82	−2.[illegible]4	−[illegible].41	[illegible].06
S.D	4 65	4.28	4.38	4 40	6.93	6.50	9 16

In dB re 0 hearing level (ISO 1964).

The effect of the ambient noise level in the testing room can be seen on the low frequencies in Table II Since the high frequency thresholds are close to 0 dB hearing level, it seems reasonable that the low frequencies should also be near 0 dB hearing level. Thresholds appeared to be worsened by about 11 dB at 125 Hz, 15 dB at 250 Hz, 18 dB at 500 Hz, and 6 dB at 1000 Hz. This does not seem unreasonable given the ambient noise levels reported earlier Even with this level of "masking present however Table III shows that the cold exposure did affect the air conduction thresholds particularly in the lower frequencies (from 125 Hz through 500 Hz) following the 20 min cold exposure. Thresholds were very slightly improved (though not significantly so) in the higher frequencies (from 2000 Hz through 8000 Hz)

The mean air-conduction shift found was statistically significant at 125 250 500 and 1000 Hz. The mean threshold shifts found from 125 Hz through 1000 Hz were all in the direction of poorer hearing whereas at the higher frequencies the shifts, although not statistically significant, were all in the direction of slightly improved hearing. This type of audiometric curve, with depressed low frequency air-conduction thresholds, when accompanied by normal bone-conduction thresholds, is usually considered to be representative of increased stiffness in the mechanical action of the ear (Campbell, 1950)

An operational definition was established for determining which of these subjects should be classified as "shifters" in air-conduction thresholds following the cold exposure. It was decided that a subject would be classified as a shifter if two of the three low frequency thresholds (125 Hz, 250 Hz, and 500 Hz) shifted by +4 dB or more and the third shifted by at least +2 dB In this way 20 subjects (10 males and 10 females) were found to have air-conduction thresholds that were

Table III *Means, standard deviations and significance levels of mean air-conduction threshold shifts found for the 46 subjects following a 20 min cold exposure*

	Frequency (Hz)						
	125	[illegible]50	500	1 000	2 000	4 000	8 000
Mean[a]	3 17	2.52	1 83	0.91	−0.[illegible]6	0.09	−0.61
S.D	4.21	3 30	3 70	2.37	2.75	3 40	3 67
Significance level of mean shift	0 0005	0.0005	[illegible]0.00	0.01	0.5	0.86	0.[illegible]7

In dB re pre-exposure threshold

shifted downward by the cold exposure. Fig. 1 displays the pre- and post-exposure air-conduction thresholds for the shifter group. Fig. 2 presents the same data for the remaining 26 subjects classified as non-shifters. The threshold shifts found in the shifter group were greatest in the low frequencies (in the direction of poorer hearing), disappeared in the mid frequencies and became very slightly improved in the high frequencies. The non-shifters, on the other hand, showed virtually no threshold shifts at any frequency and the variation around zero appeared to have no pattern. The differences in the air-conduction shifts between the two groups were significant at 125 Hz, 250 Hz, and 500 Hz (at a level of 0.0005), whereas none of the pre-exposure thresholds were significantly different from those found for the total group. When the threshold shifts were correlated with pre-exposure thresholds at 125 Hz and 250 Hz, they were significantly related and there was a marginal correlation at 500 Hz. In all three cases the relationship was negative indicating that the better the original threshold at these frequencies, the greater the size of the threshold shift.

The ranges, means, and standard deviations

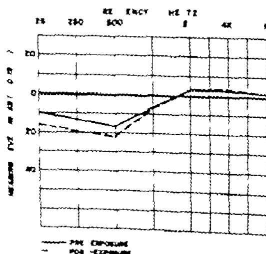


Fig. 1. Audiogram showing the mean pre- and post-exposure air-conduction thresholds for the shifters (N = 20).

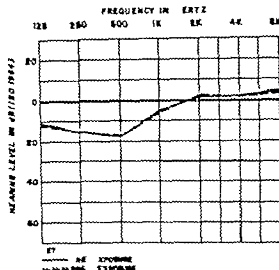


Fig. 2. Audiogram showing the mean pre- and post-exposure air-conduction thresholds for the non-shifters (N = 26).

of the pre- and post-exposure tympanic temperature readings and shifts in tympanic temperature as well as the significance level of the mean shift is given in Table IV. The mean pre-exposure temperature was 98.00 F which agrees well with the figure reported by Ganong (1967) (98.10 F) for healthy young adults. The mean post-exposure temperature reading was 97.28 F showing a mean shift of −0.72 F. In no case did tympanic temperature rise following the cold exposure although in a few cases it was unchanged. There was a slight difference in the pre-exposure temperatures of the shifters and non-shifters.

Table IV. *Ranges, means and standard deviations of the pre- and post-exposure tympanic temperature readings and shifts in tympanic temperature for the total group of 37 subjects*

	Range (°F)			
	Minimum value	Maximum value	Mean (°F)	S.D. (°F)
Pre-exposure	96.70	99.50	98.00	0.35
Post-exposure	95.40	98.40	97.28	0.68
Temperature shift	2.40	0.00	−0.72*	0.55

* Significant at less than the 0.0005 level.

Table V *Ranges means and standard deviations of the pre- and post-exposure middle ear pressure readings and the shift in middle ear pressure and significance level of the shift for the total group shifters and non-shifters*

	Range[a]			
	Minimum value	Maximum value	Mean[a]	S.D
Total group ($N=11$)				
Pre-exposure	0	30	8.64	10.02
Post-exposure	10	40	19.55	11.28
Shift	0	25	10 91	7.35
Shifters ($N=6$)				
Pre-exposure	0	10	1 67	4 08
Post-exposure	10	35	15 00	10.00
Shift	10	25	13 33[b]	6.06
Non-shifters ($N=5$)				
Pre-exposure	10	30	17.00	8.37
Post-exposure	10	40	25 00	11 18
Shift	0	20	8.00[b]	8.37

In mm water pressure.
[b] *Difference between shifters and non-shifters is not* statistically significance ($P=0.25$).

The mean pre-exposure temperature for the shifters was 97 83 F and for non shifters was 98.15 F The mean temperature shift was virtually the same for both groups being −0 68 F for the shifters and −0 75 F for the non-shifters.

With regard to acoustic impedance Hood and Lamb (1969) reported a mean of 1565 ohms with a standard deviation of 593 and a range of 657 to 2964 ohms using a sample of 42 normal ears. Some of this variation was undoubtedly due to the depth of insertion of the eartip In order to test this notion two post-exposure impedance readings were made for five of the subjects once using an insertion done in the normal manner and once with the eartip inserted to the point at which the pre-exposure volume had been matched. The former shall be referred to as free impedance and the latter as controlled impedance The differences between controlled and free impedance readings for the five subjects were within normal test retest variability Hood and Lamb (1969) reported a mean test retest variation of 130 ohms with a range of 4 to 347 ohms. Measurements of acoustic impedance in the plane of the eardrum were made on 44 out of the 46 subjects free impedance measurements on 32 and controlled impedance measurements on 17 The mean pre-exposure impedance levels agree quite well with the Hood and Lamb data. All of the shifts in impedance were in a positive (stiffer) direction following the cold exposure regardless of whether the post-exposure measurements were controlled or free. The total amount of the shift found however following the exposure was not significant for either the controlled or the free impedance ($P=0.23$ and $P=0$ 12, respectively).

The 20 min exposure had no significant effect on overall acoustic impedance and differences between shifters and non shifters were small and not significant with regard to overall acoustic impedance This is probably because this measure is too gross for our purposes.

When middle ear pressure measurements were obtained pre and post-exposure on a random sample of subjects representative of the total group shifters and non shifters, some differences were found The measurement taken here is the amount of air pressure that must be added to the external auditory canal in order to reach the point at which the ear canal and middle ear pressures are equal and the eardrum is in the midline position In other words, it is the reading of the equivalent middle ear pressure. The ranges, means, and standard deviations of the pre and post exposure middle ear pressure readings and the shift in middle ear pressure following exposure and the significance of this shift is given in Table V for the total sample and for the shifters and non shifters.

Viewing the total group Table V reveals that there was a real difference in middle ear pressure from pre-exposure to post-exposure in that the cold exposure apparently caused the middle ear pressure to increase by more

than 10 mm water pressure. This shift in middle ear pressure is significant at the 0.001 level of confidence. However both the shifters and non-shifters showed this increase in middle ear pressure following exposure. Although the shifters showed a slightly larger increase, it was not statistically significantly different from the shift shown by the non-shifters. Prior to the exposure, however the shifters middle ear pressure was significantly lower than that of the non-shifters. The pre-exposure mean for the shifters (1.67 mm) was close to what would be considered the mean normal reading (0 mm) and well within the range reported by Hood and Lamb (1969) −5 mm to +5 mm. The non-shifters, on the other hand, had greater middle ear pressure (17.00 mm) prior to exposure. The implication is that the non-shifters ears were stiffer prior to exposure but then moved in the same direction as the shifters ears moved.

In addition, it is helpful to know the compliance readings at various pressure settings so that the relationship between pressure and compliance can be determined. In the present study the pressure was varied in 40 mm steps from +200 mm water pressure to −200 mm water pressure. The balance meter on the acoustic bridge was set at +10 for the condition of +200 mm water pressure and its readings then noted for each of the other conditions. The lower the balance meter readings, the more compliant the system would be. One would therefore expect, if one were to draw a curve using these points from +200 mm water pressure to −200 mm water pressure, to have the curve peak at 0 mm water pressure since this should be the point of greatest compliance in the normal ear. Fig. 3 displays the mean compliance readings (again using a random sample of the subjects) at each of the pressures tested for the total group (15 subjects) for the shifters (7 subjects) and non-shifters (8 subjects) in the pre and post exposure conditions.

Again differences in pre-exposure readings were found between the shifters and non-

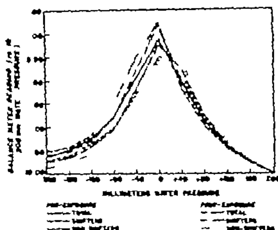


Fig. 3. Mean pre- and post-exposure compliance readings at each of the pressures tested for the total group, shifters and non-shifters.

shifters. From the pre-exposure curves in Fig. 3 it can be seen that, at most pressure levels the non-shifters ears were less compliant than the shifters ears. This agrees with the findings cited earlier. A difference appears to exist between the shifters and non-shifters' ears prior to the cold exposure. All of the various impedance measurements indicated that shifters had more compliant ears to start with. The implication is that the cold only affected hearing thresholds in ears with normal compliance. Threshold shifts did not occur in those ears that were already stiffer than normal (by about 15 mm water pressure) *prior* to the exposure. However both of the groups (shifters and non-shifters) compliance readings reacted similarly to the cold exposure. The compliance curve in both cases seems to have been shifted with the result that compliance under negative canal pressure conditions appears to be reduced following the cold exposure and slightly enhanced under positive canal pressure conditions. This is consistent with the previous finding concerning the change in middle ear pressure following the cold exposure. The implications of this finding will be discussed later. The post-exposure curves for the total group shifters and non-shifters appear to have been shifted to the

right by approximately 10 mm water pressure the change in middle ear pressure found following the cold exposure

The three types of impedance measurements discussed so far all resulted in the same conclusions 1) all of the subjects appeared to be affected in much the same way and to the same extent following the cold exposure and 2) the subjects whose hearing thresholds did not shift following the exposure had stiffer ears prior to exposure.

The last type of impedance measurement made was concerned with the intra-aural stapedius muscle reflex threshold to a stimulus of 250 Hz. The mean results were approximately the same for all of the groups both before and after the cold exposure and the level found agrees well with previous research. Jepsen (1963) reported mean thresholds of hearing and stapedius reflexes by frequency according to the age of the subjects. He found the reflex threshold for a stimulus of 250 Hz for 20 year old subjects to be approximately 85 dB above threshold (and normal threshold at 250 Hz to be 0 dB) If one assumes that the threshold for the groups of subjects tested here would have been approximately 0 dB if they had been tested under ordinary audiometric testing conditions, i.e. in a sound-treated room, one would conclude that the muscle reflex thresholds found in this study agree closely with those found by Jepsen (82 dB as compared with 85 dB). The results imply that the subjects had normal hearing at 250 Hz, and that differences in middle ear pressure and degree of stiffness of the system had no effect on the intra aural muscle reflex threshold when a 250 Hz stimulus was used.

Experiment II
Varying Exposure Durations and Recovery

The purpose of the second experiment was threefold 1) to check the reliability of the effects of the previous 20 min exposure on pure tone thresholds, middle-ear impedance and tympanic temperature 2) determine what the critical length of exposure is before hearing thresholds are affected and 3) allow for recovery tracking Nine of the 20 normal hearing young adult subjects who had been previously classified as shifters returned for three additional testing sessions with at least 24 hours but not more than four days between each session These sessions differed in the length of cold exposure—one being of five minutes duration one of ten minutes duration and the third a repeat of the 20 min duration The exposures are identified as follows Exposure 1 initial 20 min exposure reported in Experiment I Exposure 2, second 20 min exposure Exposure 3 10 min Exposure 4 5 min At each of these latter three testing sessions all procedures were followed exactly as described in Experiment I above with the exception that bone-conduction thresholds were not obtained. The order of the three exposures was rotated.

In addition in order to examine recovery patterns, pure tone air-conduction thresholds were obtained in the test ear for each subject immediately following the cold exposure and then every 20 min until the thresholds returned to normal and became stable (Thresholds were considered stable when measured no less than two times at 20 min intervals with no changes observed ±2 dB) Finally tympanic temperature and all impedance measurements were made once more for each subject as soon as air-conduction thresholds had stabilized at pre-exposure levels. The purpose of this final check was to determine if impedance readings and tympanic temperature had also returned to normal (pre-ex

Table VI. *Means and standard deviations of the air-conduction threshold shifts at each of the three exposure durations, plus significance levels as a function of exposure duration.* ($N=9$)

Frequency (Hz)	Means (min)[a]			Standard deviations (min)[a]			Significance level
	20	10	5	20	10	5	
125	6.00	2.89	0.67	2.83	2.01	3.16	0.001
250	3.11	4.00	1.33	2.03	2.83	2.45	0.01
500	4.67	2.22	1.11	3.16	3.38	3.33	0.08
1 000	2.22	0.00	0.00	2.54	2.24	2.00	0.08
2 000	−0.89	−0.67	−1.11	2.03	2.00	2.47	0.91
4 000	0.44	1.11	−2.00	1.33	2.03	2.24	0.24
8 000	−0.67	1.56	0.22	1.73	1.67	2.11	0.15

[a] In dB re pre-exposure thresholds.

posure) levels when air-conduction thresholds had done so.

RESULTS

Analysis of [v]ariance showed that the pre-exposure air-conduction thresholds for each of the three exposures, 20 min, 10 min and 5 min did not differ significantly ($P \geq 0.15$) from one exposure to another at any frequency. The means and standard deviations of the threshold shifts for each of the three exposure durations is presented in Table VI. The length of the exposure duration has a statistically significant effect on the size of the threshold shift at 125 Hz, 250 Hz, and approaches significance at 500 Hz and 1000 Hz. By 2000 Hz the length of exposure no longer appears to have any significant effect on the size of the threshold shift. It appears that there is a real difference in threshold shift particularly in the lowest frequencies, depending on the length of exposure. Thus, the longer the exposure the greater was the low frequency air-conduction threshold shift.

The mean pre-exposure tympanic temperature (97.44 F) was slightly below that found for the total group of 46 subjects (98.0 F), but agreed quite well with the mean pre-exposure temperature found for the shifters (97.83 F), the group from which the nine were drawn. The pre-exposure differences from Exposure 1 to Exposure 2, 3 and 4 were not significantly different. However the temperature shift found following the second 20 min exposure was larger (−1.46 F) than that found for the shifters following the first 20 min exposure (−0.68 F). The temperature shifts found following the 10 and 5 min exposures were similar to each other and to the level found originally following the 20 min exposure. Exposure of the ears, then, for even 5 min to a temperature of approximately −7 F affects the temperature on the tympanic membrane. There appears to be no distinct relationship between the length of exposure and the size of the shift. The temperature shifts following all of the exposure durations were approximately the same with the exception of the second 20 min exposure duration.

As in the case of the 46 subjects, none of the differences found in pre- or post-exposure acoustic impedance were significant, nor were the shifts found significant. Either there was no change in absolute impedance following the cold exposure or this measure is too gross for the small effects being considered here. In viewing middle ear pressures, however differences do appear as they did among the group of 46 subjects in Experiment I. Except for the 10 min exposure, a real difference in middle ear pressure of about +10 mm was found again following the cold exposures. Provided that the cold exposure is at least 5 min, the length of the exposure probably has no effect on the size of the shift. The

Table VII. *Mean air-conduction thresholds by frequency following the 20 min exposure and then at 20 min intervals* ($N = 9$)

	Frequency (Hz)						
	125	250	500	1000	2000	4000	8000
T[b]	8.67[a]	12.44[a]	13.56[a]	1.44[a]	−7.33[a]	−9.66	−10.56
T_{20}	6.86	10.96	11.63	2.82	−7.49	−9.28	−8.99
T_{40}	5.09	8.29	10.52	2.60	−7.71	−9.95	−9.66
T_{60}	3.53	8.07	9.19	1.04	−7.27	−9.73	−10.32
T_{80}	3.31	8.07	8.52	1.26	−7.05	−9.73	−9.44
T_{100}	3.31	8.06	8.52	1.49	−7.05	−8.62	−9.44

[a] In dB re 0 hearing level (ISO 1964).
[b] *The intervals are labeled T* (threshold immediately following the exposure), T_{20} (threshold after 20 min of recovery), T_{40} etc.

peculiar shift found following the 10 min exposure is difficult to explain but is most likely due to a couple of subjects who reacted peculiarly to this particular exposure and thus represents extreme values.

There were no significant differences found between the pre-exposure compliance measurements prior to any of the three exposure sessions. There were, however significant differences found in the compliance shifts following each of the three exposure durations for the nine subjects. The shifts became significantly different according to the length of the exposure under positive canal pressures only The pre and post-exposure curves for the 20 and 5 min exposures are very similar and like the pre and post-compliance curves found for the total group of subjects. The 10 min exposure compliance curves, however are different from the others. The shift in direction is directly related to the shift in direction found in the middle ear pressure for this exposure duration. Whatever caused the pressure changes must have caused the compliance changes also and cannot be explained. These results are out of keeping with the air conduction results obtained. (The air-conduction shifts found following the 10 min exposure were intermediate between the 20 and 5 min exposures.)

The differences in pre and post-exposure compliance readings for the 20 min and 5 min exposure durations were small but all in the direction of greater stiffness following the 20 min exposure at both negative and positive pressures. The compliance curve then as was the case in the 46 subjects, appears to be the most sensitive measure of the effect of the cold exposure on the ear other than the shift in hearing threshold. The variation in length of the cold exposure seems to affect the extent of the shift in compliance namely the longer the exposure duration the greater the stiffness of the middle ear

The last measure of acoustic impedance examined was the intra aural muscle reflex threshold to a stimulus of 250 Hz. The pre-exposure stapedius muscle reflex levels were normal and very similar to those found for the total group of shifters (82.5 dB) The difference in intra aural stapedius muscle reflex thresholds following the three exposures were not significant. Again, the implication is that the intra-aural muscle reflex threshold to a stimulus of 250 Hz is not affected by the cold exposure and not related to shifts in compliance of the ear or to air-conduction threshold shifts.

Pure tone air-conduction thresholds were obtained for each of the nine subjects immediately following each of the three exposures and then at 20 min intervals until the thresholds returned to normal Table VII presents the mean post-exposure air-conduction thresholds by frequency at 20 min intervals following the 20 min exposure duration

Recovery is defined as the point in time when thresholds returned to normal (pre exposure) levels (± 2 dB) and does not include the final or stabilization check thresholds. Recovery appears to proceed rather systematically from T to T_{60} and is then essentially complete within 60 min following the conclusion of the exposure. Of the nine subjects tested one took only 20 min for full recovery one took 40 min, six took 60 mm, and one took 80 min.

Individual recovery curves for the frequencies 125 Hz, 250 Hz, and 500 Hz are presented in Fig. 4 for the nine subjects following the 20 min exposure. As can be seen, the three recovery curves are quite similar (with the exception of 250 Hz at T_{40}) and the rate is fairly constant. The point of complete recovery should ideally be the time at which the recovery curve reaches the 0 point. However there is a certain amount of test-retest variability in threshold measurement and taking this into account, it appears that the air conduction threshold shift at 125 Hz, 250 Hz, and 500 Hz following a cold exposure of 20 min has fully recovered within one hour after the exposure

Full recovery following the 10 min exposure took place within 40 min following the conclusion of the exposure. Of the nine subjects, seven showed full recovery after 40 min,

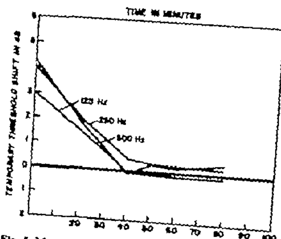


Fig. 5. Mean recovery curves at 125 250, and 500 Hz following the 10 min exposure duration (N = 9).

one following 60 min, and one subject did not show any threshold shift at all. The individual recovery curves for the frequencies 125 Hz, 250 Hz, and 500 Hz following the 10 min exposure duration are shown in Fig. 5 Although the starting points are somewhat lower than in Fig. 4 (the threshold shift following the 20 min exposure), the recovery curves are again quite similar in rate as well as pattern.

Recovery following a 5 min exposure cannot be examined, since there were no significant threshold shifts following this length of exposure. It appears that the pattern of recovery from temporary threshold shifts caused by cold exposure are similar for varying lengths of exposure but that the length of the exposure determines the time required for recovery

The tympanic temperature readings returned to pre-exposure levels within the amount of time needed for the air-conduction thresholds to recover

The differences between the pre-exposure and final acoustic impedance measurements were also very small. The mean difference from pre-exposure to final reading was −2.78 ohms for the 20 min exposure. Middle ear pressure, on the other hand, did not completely return to pre-exposure levels by the

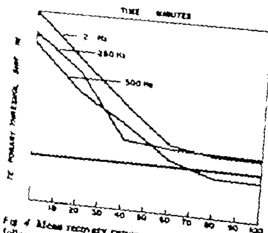


Fig. 4. Mean recovery curves at 125 250, and 500 Hz following the 20 min exposure duration (N = 9).

time of the final measurement. The mean middle ear pressure reading prior to the 20 min exposure was −7 1 mm water pressure, and by the time of the final check it was +1 2 mm water pressure The question exists as to whether some of this difference is due to test retest variability or whether perhaps the middle ear pressure had not fully recovered by the time the air-conduction thresholds had done so

There were differences between the pre-exposure and final compliance readings also The ears apparently were somewhat more compliant at all pressures at the time of the final check but it should be noted that the differences are approximately equal at all of the pressure levels. The type of shift in compliance readings found following both 20 min exposures, however was completely gone by the time of the final compliance check. Finally the acoustic reflex was unaffected by the cold exposure and remained unchanged at the time of the final testing.

Thus, it appears that the effect of cold exposure on impedance is also temporary and that the shifts found essentially recover within the time necessary for the air-conduction thresholds to recover

Conclusions

Cold exposure as described herein appears to have a specific effect on low frequency pure-tone air-conduction thresholds, middle ear impedance, and tympanic temperature When the test conditions were replicated, the same results, in general, were obtained. Further it was found that 10 min exposure was sufficient to affect the hearing mechanism but that 5 min exposure was not. The critical exposure duration then is somewhere between 5 and 10 min.

The recovery curves following the 20 and 10 min exposures were similar in rate and pattern Recovery from the 20 min exposure took approximately one hour whereas recovery from the 10 min exposure took about 40 min

Discussion

Five possible explanations may account for the threshold shifts found. These are 1) the shift in threshold is due to the fact that the cold affects the entire body not only the ear resulting in generalized chilling shivering etc. 2) there is a direct effect of the cold air on the action of the inner ear 3) there is a direct effect of the cold air on the action of the middle ear muscles, tendons, and ligaments 4) the threshold shifts are due to a disequilibrium in air pressure between the external canal and the middle ear following the cold exposure and 5) there is a direct effect of the cold air on the action of the tympanic membrane Although it is unlikely that any single explanation will account for the effects found each will be considered individually

In order to determine what effect generalized body chilling might have on hearing thresholds, three subjects who were lightly dressed were exposed for longer periods of time to somewhat higher temperatures, 35 to 40 F Each of these subjects remained in this environment for 30 to 40 min or until they were thoroughly chilled as evidenced by shivering and horripilation None of these three

subjects showed any shift in hearing following this exposure. The subjects in the present study were, of course, warmly dressed, but with their heads exposed the possibility existed that the shift in hearing thesholds might have been caused by generalized body cooling or by muscular contractions throughout the body rather than by specific cooling of the head only. The pilot study referred to above demonstrated that thoroughly chilling subjects does not have any effect on hearing thresholds. This does not rule out the specific effect of cold on the ear itself however.

As discussed in the review of the literature, Kahana, Rosenblith, and Galambos (1950) and Gulick and Cutt (1960) demonstrated that when body temperature was reduced the magnitude of the cochlear response was likewise reduced, and Gulick and Cutt also reported concomitant hearing losses. But, it is also well established that the inner ear can be affected by cold air even when body temperature is unchanged. This, of course, is what occurs in caloric testing, whether cold water or cold air is employed as the stimulus. The question, therefore of whether the threshold shifts found following the cold exposure might be due to changes in the inner ear is certainly a pertinent one.

One would expect, however that were these threshold shifts due to changes in the inner ear the bone-conduction thresholds would also have been affected. Since bone-conduction thresholds remained essentially the same following the cold exposure, one must conclude that the shifts in air-conduction thresholds following the cold exposure were probably due to factors other than changes in the response of the inner ear. The conclusion must be made cautiously since it is possible that given longer exposure durations or lower temperatures, an effect on bone-conduction thresholds might occur as Carco (1952) reports. This will be discussed in more detail later in conjunction with middle ear pressure.

The possibility needs to be explored that the middle ear became so cold following the exposure that the tensor tympani and stapedius muscles contracted, thereby creating the air-conduction loss. Since no threshold shift was found when the pilot study subjects were put into the cold room lightly dressed and were thoroughly chilled and shivering, it seems unlikely that the middle ear muscle contractions can be credited with the threshold shifts found following the exposure under the present conditions. Also, even if muscular contractions had occurred, it is improbable that duration of contraction would have extended for up to an hour post exposure, the length of time necessary for full recovery following a 20 min exposure. It is possible however that there was some contraction of the tendons and ligaments in the middle ear most particularly the tendons of the stapedius muscle which might have contributed in part to the hearing threshold shifts found and to the overall increased impedance of the middle ear system.

Smith (1943) mechanically fixated the stapes in cats and measured changes in the cochlear responses for air and bone-conduction which resulted. The degree of stapes fixation varied according to the amount of tension placed on it. Hearing losses found were greatest in the low frequencies by air-conduction the mean losses ranging between 30 and 40 dB, whereas by bone-conduction the losses ranged only between 9 and 10 dB. If there were some cooling of the tendons of the stapedius muscle, one might expect to get similar results, i.e. hearing loss in the low frequencies by air-conduction and little or no hearing loss by bone-conduction.

The air-conduction losses found here, however were much smaller than those found by Smith, implying that if increased tension in the stapedial muscle was responsible for the threshold shifts found in this study the increase in tension must have been quite small.

The greatest effect of the cold air exposure would be expected in the outer ear canal. Pre and post-exposure otoscopic examinations were made by an otolaryngologist on a sample

time of the final measurement. The mean middle ear pressure reading prior to the 20 min exposure was −7 1 mm water pressure and by the time of the final check it was +1 2 mm water pressure. The question exists as to whether some of this difference is due to test retest variability or whether perhaps the middle ear pressure had not fully recovered by the time the air-conduction thresholds had done so

There were differences between the pre exposure and final compliance readings also The ears apparently were somewhat more compliant at all pressures at the time of the final check but it should be noted that the differences are approximately equal at all of the pressure levels. The type of shift in compliance readings found following both 20 min exposures, however was completely gone by the time of the final compliance check. Finally the acoustic reflex was unaffected by the cold exposure and remained unchanged at the time of the final testing

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Using the Valsalva procedure Loch (1942) found that a moderate increase in middle ear pressure reduced hearing acuity in the lower frequencies by approximately 10 dB. Wever and Lawrence (1954) believed that these effects were probably due to altered tension of the tympanic membrane. They further suggested, however that should the pressure in the middle ear be sufficient, the effect might continue on into the inner ear One would suspect that in the present investigation the pressure build-up in the middle ear was not sufficient to affect the inner ear since the bone-conduction thresholds were unchanged. Based on the specific effects of both positive and negative pressures on air-conduction thresholds, they concluded that both the damping and stiffness of the tympanic membrane were affected, the damping being responsible for the reduction in sensitivity and the stiffness causing the low tones to be affected primarily

The possibility exists that the tympanic membrane is affected by the cold air reaching it through the external auditory canal. If the tympanic membrane were to be cooled, the result would be increased stiffness in its action. In order to demonstrate whether or not the tympanic membrane actually is cooled by the cold exposure, each individual's temperature was taken on the tympanic membrane both before and after each exposure session. The temperature of the tympanic membrane was decreased by approximately 0.7 F following the cold exposure. It is unknown to what degree the membrane's action was affected, but, should the tympanic membrane be stiffened as a result of the cooling, the change in impedance would result in depressed low frequency air-conduction thresholds. When the pure-tone air-conduction thresholds had returned to normal so had tympanic temperature This must certainly be considered as part of the explanation for the threshold shifts observed.

In conclusion, five possible explanations have been presented in order to account for the air-conduction threshold shifts found. The first, that the effects seen were due to generalized body chilling and/or overall muscular contractions, seems unlikely The second, that the effects were due to changes in the action of the inner ear seems unlikely also. However it is felt that this could be a factor were the exposure longer or the cold more extreme. The most likely explanation for the threshold shifts found appears to be some combination of three physiological effects, namely 1) subtle changes in the action of the middle ear muscles, 2) increased air pressure in the middle ear and/or 3) a reduction in the vibratory capacity of the tympanic membrane.

The combination of one or more of these factors would result in an increase in the stiffness of the middle-ear system. This change in impedance of the system would be reflected in the post-exposure depressed low frequency pure-tone air-conduction, threshold shifts found. Since the type of audiogram obtained following the cold exposure is similar to that found in patients with adhesive otitis media and pre-clinical otosclerosis, care should be taken to determine how long a patient was out of doors in cold air temperatures immediately preceding an audiometric evaluation. Sufficient time should be allowed for threshold shifts caused by the cold air out doors to fully recover before pure-tone testing is begun to help insure valid pure tone test results and to aid in avoiding misdiagnoses of middle ear pathology

Finally this investigation provides further information regarding the effects of altering the normal physiological state of the human middle ear system.

of the subjects. He found the walls of the canal definitely cooled by the exposure, and the resultant stiffening of the canal walls to be very noticeable. It can be demonstrated by use of the tympanic thermometer that the tympanic membrane is also affected by the cold exposure. It does not seem unreasonable to further assume that the middle ear is somewhat cooler than normal also but undoubtedly not as cool as the external canal. Air of course, expands as temperature rises and should the temperature in the middle ear be warmer than that in the external auditory canal a disequilibrium in air pressure between the external canal and the middle ear cavity would result. Under ordinary circumstances, one would expect that this difference in air pressure would be equalized through the action of the eustachian tube. For a variety of reasons the eustachian tube may not have been capable of correcting these pressure differentials under the experimental conditions used in this investigation. First, a certain minimum amount of pressure difference is necessary to open the eustachian tube probably between 0.5 and 4 mm mercury (Ballenger 1969). The increase in air pressure in the middle ear found in this investigation i.e. approximately +10 mm of water pressure (10 mm water pressure is equal to 0.7 mm mercury) simply may not have been enough to blow open the eustachian tube. However it may have been enough to cause a slight change in the mechanical action of the middle ear

Secondly it was noted that many of the subjects emerged from the cold room with "sniffles. Scott-Brown (1965) and Boies (1957) also comment on temporary congestion, inflammation membrane swelling and nasal discharge as a result of chilling. This could certainly have reduced the patency of the eustachian tube as would happen in the case of a common cold. It is more difficult to equalize pressure between the middle ear and outer ear when there is mucosal congestion. In fact, the possibility exists that the patency of the eustachian tube may be the key factor that differentiated subjects who shifted from those who did not shift. Those subjects whose eustachian tubes remained most patent were perhaps more able to compensate for the disequilibrium in air pressure and therefore did not show the threshold shift whereas, those subjects who were not able to equalize the pressure between the middle and outer ear showed the threshold shift. This explanation agrees with our findings cited earlier: following the cold exposure the middle ear pressure had increased more in the shifters than in the non-shifters. Presumably the non-shifters were able to equalize the air-pressures in their external canals and middle ears, thereby maintaining normal impedance and normal air-conduction thresholds. The differences are small, however as is the number of subjects involved. More important perhaps is the fact that most of the subjects showed some increase in the middle ear pressure following the exposure. This shift in middle ear pressure following the cold exposure was significant at the 0.001 level of confidence.

It was noted previously that post-exposure compliance curves showed reduced compliance under negative canal pressure conditions and enhanced compliance under positive canal pressure conditions. If the middle ear pressure had indeed been somewhat increased and the tympanic membrane displaced outwards slightly this is exactly what one would expect to occur. Adding pressure to the external canal would help to bring the air pressure back into equilibrium and allow the tympanic membrane to come closer to its normal (mid-line) position, thereby reducing the stiffness (and increasing the compliance) of the system. Withdrawing air from the external canal creating a negative canal pressure, would simply make the disequilibrium even greater resulting in an even stiffer (less compliant) system. It seems reasonable to assume that this is what did occur and that this accounts for the post-exposure compliance curves found.

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Summary

In Experiment I forty-six subjects were exposed to a cold air temperature of −7 F for 20 min while warmly dressed but with their head and ears exposed Pure tone air and bone-conduction thresholds, various impedance measurements, and tympanic temperature were obtained before and after exposure. In Experiment II nine subjects were exposed to the same cold temperature condition but on three occasions for 20 10 and 5 min The results indicated that bone-conduction thresholds were not affected by cold exposure Air-conduction thresholds however were depressed in about half of the subjects following the cold exposure The longer the exposure the greater was the threshold shift found. These subjects also demonstrated post exposure increased middle ear pressure and decreased tympanic temperature. The threshold shifts were probably due to increased middle ear impedance. Recovery took approximately one hour following the 20 min exposure and 40 min following the 10 min exposure. The 5 min exposure did not affect pure-tone thresholds.

Since the type of audiogram obtained following the cold exposure is similar to that found in patients with certain types of mild conductive hearing impairment, it is important to determine how long a patient was outdoors in cold air temperatures immediately preceeding audiometric testing.

Zusammenfassung

Im ersten Experiment wurden 46 Personen warm gekleidet, aber mit exponiertem Kopf und Ohren für 20 Minuten einer kalten Luft temperatur von −7 Fahrenheit ausgesetzt Vorher und nachher wurden die Schwellen werte für Reinton- (pure-tone) Luft und Knochenleitung sowie verschiedene Impedanzmessungen und die Trommelfelltempera tur bestimmt Im zweiten Experiment wurden 9 Personen derselben kalter Temperatur dreimal ausgesetzt für 20 10 und 5 Minuten Die Ergebnisse zeigten an dass die Knochenleitungsschwellen von einer Kälteexponierung nicht beeinflusst wurden. Die Luftleitungsschwellen jedoch waren bei etwa der Hälfte der Personen nach der Kälteexponierung ge senkt Je länger die Exponierung umso grösser war die aufgefundene Schwellenvershiebung. Diese Personen wiesen nach der Kälte exponierung auch einen erhöhten Mittelohr druck und eine erniedrigte Trommelfelltemperatur auf Die Schwellenverschiebungen waren höchstwahrscheinlich auf eine erhöhte Mittelohrimpedanz zurückzuführen. Die Rückbildung nach 20-minütiger Exponierung dauerte etwa eine Stunde und die nach 10 Minuten etwa 40 Minuten Die 5-minütige Exponierung hatte auf die Reinton- (pure tone) Schwellen keinen Einfluss. Da die Art Audiogramm, die man nach Kälteexponierung erhält derjenigen von Patienten mit gewissen Arten von leichter Leitungshorschwäche ähnelt, ist es wichtig zu bestimmen wie lange unmittelbar vor einer audiometrischen Untersuchung der Patient im Freien in kalter Lufttemperatur war

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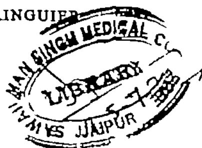
SUPPLEMENT 291

Disorders of the Auditory Apparatus Caused by Embryopathy or Foetopathy: Prophylaxis and Treatment

BY

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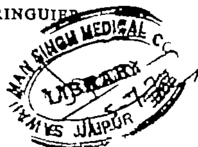
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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 291

Disorders of the Auditory Apparatus Caused by Embryopathy or Foetopathy Prophylaxis and Treatment

(*a*) Deafness as a result of congenital malformations occurring during the first three months

(*b*) Deafness resulting from diseases of the foetus occurring after the fourth month

BY

A. MORGON D CHARACHON and N BRINGUIER

Study by the Institut d'Audio-Phonologie of the Faculty of Medicine of Lyon
Director Professor Dr Med. P Mounier Kuhn

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Introduction

Within the vast sphere of the aetiology of deafness in children the particular field of the pathology of pregnancy likely to affect the normal development of the embryo and later that of the organs already differentiated, causing an impediment to their working in the future, has been studied for only a few decades and is still partly unexplored. As far as prophylaxis is concerned the study of these factors is of primordial importance. Only exact knowledge of teratogenous or noxious agents, whether they are infectious or toxic for example, can lead to progress in research for effective means of prevention.

Unfortunately at present, it is only too often so that an aetiological investigation into the case of a young malformed or deaf child shows the otologic lesion to be of intra-uterine origin.

Embryo

Before approaching the study of the factors likely to perturb the development of the ear before birth it is necessary to review its formation—paying particular attention to the origin of the tissue of each element and to the timing of their evolution, these data help to explain how a particular part of the ear is affected, according to lesion affinities and the moment at which the pathological process intervenes during pregnancy.

The outer ear is of ectodermal origin, the external auditory canal and the pavilion develop from the first branchial fissure and from the outer covering of the first and second arches which border it.

Three ectodermal buds, which later become the pavilion appear at about 43 days. Without going into the details of their development it may be said that the different reliefs of the pavilion are formed by the end of the third month, and reach their final shape between 4 and 5 months.

The tympanum is roughly shaped at about 6 or 7 weeks, the end of the external auditory canal, of ectodermal origin, comes into contact with the endoblast of the tubo-tympanic process from which it is separated by a thin layer of mesoblast. The *pars tensa* the only part formed at this stage is therefore made up of three layers, each of different embryological origin.

The Shrapnell membrane develops at about 6 months when the atticus, in its development, comes into contact with the external auditory canal. It is made up of only two epithelial layers. The tympanum then acquires its final form.

The middle ear—the tympanum and the adjacent cavities are of endodermal origin, while the ossicles are formed out of the mesoblast. The atrium and the atticus become distinct at about 10 weeks. For a long time they are full of reddish mesenchymatous tissue and do not reach their final form until the sixth

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The connections of the nerves are established between 7 and 8 weeks.

It must be borne in mind that the growth of the brain will continue up to 12 years, reaching its acme between 0 and 2 years.

Between the 6th month and birth only a few minor modifications will occur at this stage the annexes are being finished involving the tympanum a virtual cavity at this period which will not become empty until birth the Fallopian canal, the ossification of which continues, and the stapes, the volume of which is reduced as it loses its original medullar tissue.

But although anatomical and histological development is practically finished it must be stressed that histochemical modifications are still possible particularly in the case of the sensory cells of the organ of Corti, the cytochemical structure of which is finished at the end of the first month after birth.

Definitions

This information about the embryo shows how difficult it is to put a definite date on the moment during pregnancy at which pathological incidents likely to affect the developing ear occur

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The teratogenous or noxious agent may intervene in one of two ways: (i) it may have an *indirect action* the pathogenic factor not reaching the foetus.

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The Clinical Study

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Since then much work has been done in Britain (Martin, Hopkins) America (Prendergast, Beswick Warner) Switzerland (Franceschetti Bamatter and Bourquin) The first French observation was made in 1946 (Debré Saint and Thieffry)

Many statistics have been published since then in an attempt to determine the proportion of cases of deafness among the effects of rubella during pregnancy on the one hand and the proportion of cases of deafness for which rubella was responsible on the other

The figures vary considerably according to Swan rubella is responsible for deafness in 59 9% of cases according to Meyer 72.5% and according to Kuntzel 63 6% (the latter having gathered together all 1 048 cases in the world published between 1941 and 1952) when the mother suffers from rubella during the first months of pregnancy

Inquiries into the different causes of deafness in children also vary in attribution of responsibility to rubella embryopathy (9% according to Erickson 9% again according to Schwann 6 9% according to Van Gilse 1 3% according to Candiotti, 37 1% according to Hay) Studies recently undertaken at the Institut d Audiophonologie in Lyon have shown rubella to be responsible seven times out of 100 partially deaf children fifteen times in another study concerning 190 profoundly deaf children giving a total percentage of 8 4

In general the gravity of lesions caused by rubella embryopathy depends on the time of infection rather than the gravity of the infection itself

It is widely accepted that from 3 to 7 weeks it causes ophthalmological lesions, cataract and microphthalmia being the most typical and the most frequent from 5 to 8 weeks cardiovascular lesions (most frequently persistence of the arterial canal, interauricular and interventricular communication) from 6 to 9 weeks stomatological lesions

Lastly microcephalia and mental retardation are frequently associated and lower the educational possibilities when accompanying deafness even more than ocular lesions.

Some authors have even tried to establish a still more precise embryopathic timetable" in this way Brent and Tondury specify that a cataract results when the pregnant woman suffers from rubella between 29 and 35 days, that a cardiopathy regularly results between 36 and 49 days, a lesion of the inner ear between 50 and 63 days.

In fact such a timetable is not always respected, as is shown by simultaneous lesions of several organs associated ocular and auditory lesions, for example.

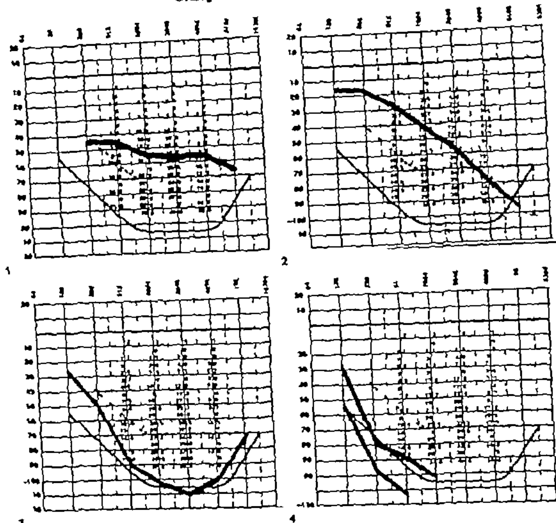
The possibility of auditory embryopathy throughout the first three months of pregnancy must therefore be admitted this is in fact the opinion of the majority

The problem of "unapparent cases of rubella in the mother must certainly be discussed but one must be very cautious about confirming such cases and insist on positive results to a serological examination of the child.

Anatomo-pathological lesions always affect the inner ear Little is known about them yet since histological observations of rubella ears are comparatively rare

Real malformations seem to be fairly rare rudimentary Corti sensory cells, underdeveloped vascular stria and tectorial membrane (Kuntzel Lindsay)

Most of the time the trouble is due to small dispersed extravasations of blood (Kelemen) haemorrhages which although very small can cause extensive damage to the inner ear in the basilar membrane or the vascular stria



Figs 1-4 Rubella embryopathy: Five types of curves (C.A.).

The posterior labyrinth is not usually affected.

In fact anatomo-pathological observations are still very sparse, and thus explains to a certain extent the multiplicity of pathogenic theories expressed: direct action of the virus, a real intracellular parasite, electively attaching itself to dividing tissues (Claverie) vascular mechanism (Schwann & Hurst, Kelemen), metabolic disturbances caused by enzyme inhibition due to the virus (Mann & Warkany).

Clinically

Rubella embryopathy is responsible for lesions of the inner ear: apart from Leicher all authors of papers are quite definite about the constant integrity of the middle and outer ear.

Perceptive deafness is generally severe, but only rarely total.

The curve of the graph may be very varied: horizontal depressed, sloping down from low to high frequencies.

Although both ears are affected, one side is generally worse than the other. Some authors have even described unilateral deafness (Jackson & Fisch) although this seems to be exceptional.

As a general rule the effect on hearing is great, severe partial deafness or profound deafness, so that even isolated the diagnosis

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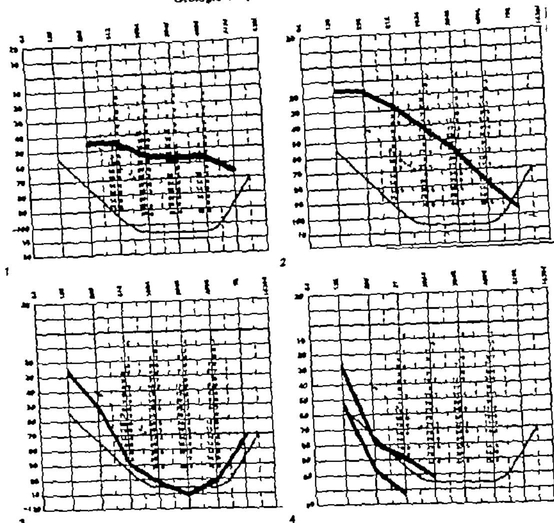
The possibility of auditory embryopathy throughout the first three months of pregnancy must therefore be admitted this is in fact the opinion of the majority

The problem of unapparent cases" of rubella in the mother must certainly be discussed but one must be very cautious about confirming such cases and insist on positive results to a serological examination of the child.

Anatomo-pathological lesions always affect the inner ear Little is known about them yet since histological observations of rubella ears are comparatively rare

Real malformations seem to be fairly rare rudimentary Corti sensory cells, underdeveloped vascular stria and tectorial membrane (Kuntzel Lindsay)

Most of the time the trouble is due to small dispersed extravasations of blood (Kelemen) haemorrhages which although very small can cause extensive damage to the inner ear in the basilar membrane or the vascular stria.



Figs. 1-4. Rubella embryopathy: Fo types of curves (C.A.).

The posterior labyrinth is not usually affected.

In fact anatomo-pathological observations are still very sparse, and this explains to a certain extent the multiplicity of pathogenic theories expressed: direct action of the virus, a real intracellular parasite, electively attaching itself to dividing tissues (Claverie), vascular mechanism (Schwann & Hurst, Kelemen), metabolic disturbances caused by enzyme inhibition due to the virus (Mann & Warkany).

Clinically

Rubella embryopathy is responsible for lesions of the inner ear: apart from Leicher all authors of papers are quite definite about the constant integrity of the middle and outer ear.

Perceptive deafness is *generally* severe, but only rarely total.

The curve of the graph may be very varied: horizontal, depressed, sloping down from low to high frequencies.

Although both ears are affected, *one* side is generally worse than the other. Some *authors* have even described unilateral deafness (Jackson & Fisch) although this seems to be exceptional.

As a general rule the effect on hearing is great, severe partial deafness or profound deafness, so that even isolated the diagnosis

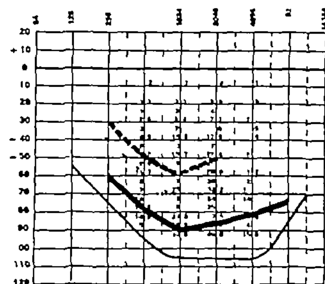


Fig 5 Congenital syphilis.
--- C O curve
—— C A curve

may be reached at an early stage by an attentive family even before lateness in beginning to speak awakens suspicion Of course the presence of other elements of the rubella traid congenital cataract or cardiac malformation *now means a systematic auditory examination* which facilitates early diagnosis of deafness.

The proof that rubella is the cause can now be provided by the search for the virus itself and above all by serological examinations of new born babies and young children Recent studies have revealed the presence of viruses in the placenta systematic research at the time of delivery would provide a conclusive argument.

Similarly deafness has been found after measles (Schachter Candiotti) influenza (Ellet Hopkins Massol) mumps (Hay Cronvald, Selander) and even poliomyelitis (Kelemen) shingles or chicken pox.

In fact, at the moment the lack of cases described makes it possible to retain these factors only as probable causes without certain confirmation because the possibility can not be eliminated.

Given the permeability of the placenta as regards most viruses and the results obtained by the innoculation of animals with viruses, logically large scale statistical inquiries should prove the influence of many viruses in the causation of auditory embryopathies.

Another argument carrying weight would be provided by evidence of the presence of the virus itself in the deaf baby or by the positive nature of specific serological reactions. Much is hoped for in this field from the research at present being undertaken by the Institut d Audiophonologie of Lyon in collaboration with the Laboratoire de Recherches Virologiques du Centre d Hygiène de l Enfance

(b) Microbic causes

Once again there are two categories of embryopathy or foetopathy those resulting from treponema now well known and those attributable to other germs.

In fact, apart from syphilis, observation are very sporadic and varied Candiotti mentions cases of deafness after diphtheria typhoid pneumonia during pregnancy Albouy mentions scarlatina in the fifth month of gestation *as a cause of deafness, also associated* with macular dysplasia.

Bernabei, Bordley Fowler also mention various microbic infections in the statistical studies they have made

Once again it is difficult to confirm the role of microbic agents as a cause of deafness. It would appear that miscarriage was more frequent than lesions of the foetus in cases of serious infections of pregnant women

On the other hand *congenital syphilis* is well known as a cause of foetopathy *its in*cidence is now limited, whereas it used to be extensive

Syphilitic infection does not cause malformation since the treponema cannot pass through the barrier of the placenta before the fifth month

There are two distinct forms (*a*) *meningoneuritis* of the acoustic apparatus, which is rare it occurs either in babies or at a later stage and is often associated with other nervous disorders, *(b) it may on the other hand,* show itself in the form of retarded labyrin

thitis, which is more frequent it affects children, especially girls, between 10 and 15 years old. it is the last stage of the Hutchinson triad (being preceded by deterioration of the teeth and interstitial keratitis).

The condition develops unevenly in spurts, affecting the ears unequally The curve of the graph is of mixed deafness at first, then osseous conduction and aerial conduction become the same.

The posterior labyrinth always suffers from hypoexcitability or inexcitability to vestibular tests. The Siegle test reveals nystagmus towards the opposite side: Hennebert's syndrome

Diagnosis is based on clinical observations, deafness is accompanied by dental and ocular deterioration (keratitis, hydarthrosis of the knees snub-nose, perforation of the velum, malformations of the long bones. Serological tests (Bordet–Wassermann and in particular Nelson) confirm it.

II PARASITE CAUSES

Although malaria haemonites are known to be able to pass through the placenta, a disorder of the ear never seems to have been described as having resulted from this condition during pregnancy

On the other hand another parasite, toxoplasma—a small protozoon—was declared responsible for embryopathy as early as 1938 by Wolf Cowen & Paige. Lesion of the brain (hydrocephaly comitiality) and eyes (characteristic dysplasia of the macula) were described in the first place. It was not until 1950 that Campbell & Clifton reported cases of deafness in children having congenital toxoplasmosis with ocular disorders. Since then other observations of auricular toxoplasmosis have been published (Dietzel, Feinmesser & Landau, Grimaud & Wayoff) and now the possibility of such a cause must not be underestimated, especially as it is a widespread condition (only 13% of the population over 30 would still have a negative serological reaction according to Lelong).

The anatomo-pathological lesions have been described by Kelemen who observed the presence of calcification, not only of the *brain* but also of the vascular stria and the spiral ligament, associated *with an inflammation* of the whole of the vestibule as well as in the middle ear

From a pathogenis point of view congenital toxoplasmosis is the result of an infection of the foetus during an illness of the mother Several points should be stressed. the parasitosis of the mother is generally not apparent, the contamination of the foetus is not constant throughout the mother's illness whenever it may occur there seems to be no risk of transmission during the first six weeks of pregnancy

Because of the size of the parasite, a lesion of the placenta is necessary for it to pass through into the foetal circulation. This lesion would occur during the parasitemic phase of the mother's toxoplasmosis.

The phase of toxoplasmic infection of the child at birth depends on the moment of infection during pregnancy (i) primary or parasitemic, characterized by icterus with hepatosplenomegaly polyadenopathies and serious visceral disorders which are *often fatal* (ii) secondary causing evolutive encephalomyelitis (somnolence, hypotonia or hypertonia, convulsions) with *modifications* of the cephalo-rachidial fluid, (iii) finally tertiary that is the phase of consequences essentially characterized by cerebral disorders (microcephalus or hydrocephaly convulsions), *ocular disorders (chorioretinitis* with the characteristic *appearance of the fundus*) and possibly auditory disorders.

Perceptive deafness, indicating lesions caused by the parasite to the neuro-sensorial elements of the labyrinth. It seems to vary in *degree: observations* published so far report either slight troubles (Dietzel) or severe deafness (Grimaud & Wayoff).

Diagnosis is at first based on clinical ex-

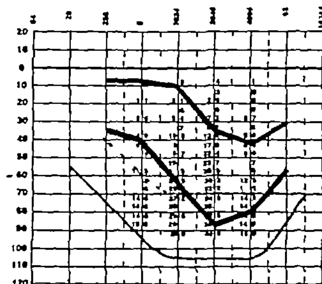


Fig 6 Congenital toxoplasmosis. Two types of curves (C.A.).

amination deafness associated with chorioretinitis, with the characteristic deterioration of the fundus, and with hydrocephaly or microcephalia with calcifications visible by means of radiography is most likely to lead to a diagnosis of congenital toxoplasmosis.

It is confirmed by serological tests showing specific antibodies. (i) either directly by action on a living strain of toxoplasma Savin's lysis test. Up to 2 years a proportion of over 1/16th is significant. After that the proportion must be over 1/128th. (ii) or indirectly by complement fixation reaction. The reaction is positive when over 1/64th.

It must, however be pointed out that because of the frequency of the illness the significance of serological tests varies according to the child's age during the first three months the presence of antibodies may be due to an infection of the mother before pregnancy. The transmitted antibodies disappear at about 3 months. It is imperative to follow the development of the proportion of antibodies as time progresses. From 3 months to 1 year positive reactions give confirmation of a diagnosis of congenital toxoplasmosis that is the only time at which serological examination can be categorical. Over 1 year positive reactions may correspond to either congenital or acquired toxoplasmosis.

The systematic use of these serological tests will probably make it possible in future to attribute to toxoplasmosis *certain* cases of congenital deafness the explanation of which otherwise remained vague. As the illness is widespread it may be responsible for more cases of deafness than have yet been estimated.

III. IONISING RADIATIONS

The dangers to the embryo and the foetus of radiation are known in general. It can cause mutation induced by its effects on the gonads, leukaemia and neoplastic processes, and morphological and functional anomalies.

Experiments with animals (Kelemen) aiming at demonstrating the behaviour of the auditory organ when the pregnant female undergoes irradiation have shown serious endo*cranial malformations with intracochlear* and pericochlear haemorrhages but without anomalies in the development of the labyrinth.

As far as human beings are concerned there is little literature specifically concerning the ear in spite of widespread inquiries undertaken in Japan for example, after the massive irradiations due to the atomic bombardments.

However on the basis of experimental data it seems advisable on the one hand to limit irradiation in the case of pregnant women and, on the other hand to bear this possibility in mind when attempting to define the cause of the aural disorders of a deaf or malformed child.

IV TOXIC CAUSES

(a) Exogenous

The dangers of medicaments and toxins to the ovum for a long time asserted by experimenters and suspected by clinicians, recognised in particular cases, has reached the forefront of interest over the past ten years since the dramatic consequences of the use of Thalidomide by pregnant women.

Many substances may pass through the placenta and damage the auditory organ. As early as 1935 Taylor said that "quinine, salicylates, alcohol pass through the placenta and are present in the foetus in the same concentration as in the mother's circulation"

In reality it is more usual to find the foetus dead *in utero* as a result of absorption of toxic substances, and until recent years few conclusive observations of deafness caused by toxic embryopathy or foetopathy had been reported. Of these it is necessary to mention auditory disorders linked with massive and prolonged doses of *streptomycin* during pregnancy as reported by Boletti & Croatto, Rebattu & Lesne.

In the study produced by the Institut d'Audiophonologie of Lyon about partial deafness already quoted, an attempted abortion at about the 10th week of pregnancy by use of quinine was the only cause of perceptive deafness of medium gravity

These medicaments seem to damage the sensory cells of the organ of Corti, but have no teratogenic effect properly speaking.

The same is not true of Thalidomide: since 1960 numerous publications have stressed the role of this substance as the cause of multiple malformations when administered during the first months of pregnancy

It is remarkable, as far as the ear is concerned, that the malformations involved not only the outer and middle ear (Partsch, Maurer Lenz, Hepp Petersen) but also the inner ear (Kleinsasser Schlothane) the two disorders being observed simultaneously in the same child. Terrahe produced a detailed radiotomographic study of these lesions in a group of 37 children from 3 to 4 years old, suffering from bilateral malformations of the outer ear-middle ear whose mothers had taken Thalidomide during the first two months of pregnancy He observed simultaneous malformations of the inner ear in 33 of them, the malformations were very varied, dysplasia of the external and superior semicircular canals; absence of all canals; rudimentary double labyrinth, absence of fenestrae, accompanying, of course, severe perceptive deafness.

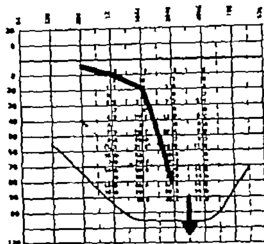


Fig. 7 Toxic foetopathy Streptomycin (C.A. curve).

Many other substances have been recognised as being responsible for a multitude of embryopathies and foetopathies, for example. cytostatics, sexual hormones, dicoumarol, derivatives of digitalis, although little is known about their effect on the ear

Synthetic antithyroid drugs should be pointed out: it is known that if they are administered to the mother during gestation they may give rise to goitre with hypothyroidism in the new-born baby

The classic forms of deafness from congenital myxoedema are known. the auditory disorder takes its place among disturbances of growth and psychomotor retardation. It does not therefore seem illogical to admit that antithyroid drugs taken by the mother cause deafness in the child. our statistics for partial deafness contain an observation where this hypothesis may be considered very probable.

Before ending this chapter on exogenous toxins, the possible role of medicaments likely to damage the inner ear by means of anoxia used during *labour and delivery* must not be neglected. *These* are volatile anaesthetics, barbiturates, neuroleptics which all pass into the foetal circulation and may have marked depressive effects on the child's respiratory

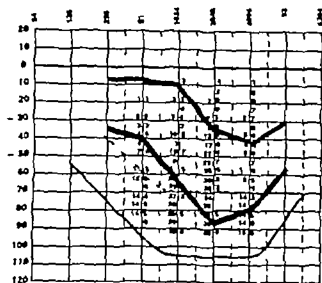


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It is of interest to refer to global statistics in order to assess the importance of embryopathy and foetopathy as causes of deafness in children.

The study of rubella embryopathy has already enabled us to consider this problem and it would be useful to take it up as a whole.

It must be said, however that most statistics do not analyse simultaneously malformations of the outer or middle ear and perceptive deafness caused by disorders of the inner ear so that it is difficult to compare the frequencies of both types.

We are more concerned with disorders of the inner ear and give two recent statistical reports from the Institut d'Audiophonologie of Lyon—one concerning partial deafness and the other profound deafness.

Embryopathy and foetopathy are responsible for 12% of cases in Table I (8% certain, 4% probable) 9.5% of cases in Table II (5.3% certain, 4.2% probable), that is 10.6% overall.

However these studies show how often the cause of deafness remains unknown (30–40%), emphasising how much more research needs to be done, particularly in the field of the pathology of pregnancy where, as we have already said, much uncertainty remains.

Table I. *Aetiology of partial deafness in young children (100 cases)*

Genic causes (22%)		
Hereditary or family deafness	22	15 certain 7 probable
Antenatal causes, embryopathy and foetopathy (12%)		
Rubella	7	6 certain 1 probable
Syphilis	1	
Toxic	2	1 certain (quinine) 1 probable (anti-thyroid treatment)
Dysgravidia (haemorrhage during pregnancy)	2	probable
Neonatal causes (23%)		
Birth trauma	1	probable
Anoxia	9	8 certain 1 probable
Nuclear icterus	6	
Prematurity	7	2 with anoxia 5 without anoxia
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Meningitis: cerebro-spinal	3	
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viral	1	
Encephalitis	4	certain 2 probable
Mumps	1	
Labyrinthic middle otitis		probable
Causes unknown (30%)		

centres, causing anoxia unless sufficient oxygenation is maintained.

It is well known that the sensory cells of the organ of Corti are particularly fragile as regards anoxia which causes widespread haemorrhages often affecting the whole of the central nervous system.

Here foetal pathology becomes bound up with neonatal pathology and in practice it is often difficult to determine the exact moment at which the child's condition began particularly as the result is the same neurological lesions of varying extent are associated with variable degrees of perceptive deafness.

(b) Endogenous

This field, which involves embryopathies with *humoral metabolic and endocrine causes* is as yet little known in any case it is often difficult to distinguish when the mother is suffering from a long-term condition existing since before the pregnancy between what is caused by the disease itself and what is caused by the treatment it necessitates. This is the case with diabetes for example a long time ago insulin hypoglycemia was found to be responsible for teratogenous effects Petersen & Tygstrup have shown that the existence of vascular lesions in the mother conditions the frequency of foetal malformations.

As far as the effect of diabetes on the development of the ear is concerned Kelemen provides interesting histological arguments anatomo-pathological examination of foetuses of 4 and 6 months (therapeutic abortion dictated by the gravity of the mother's diabetes) showed normal development of the different parts of the ear and particularly of the cochlea, but the existence of diffuse haemorrhages in the cochlear canal and the vestibulum

It would therefore seem that the characteristic feature in this case is the fragility of the blood vessels.

Kelemen made the same observation about foetuses of mothers suffering from grave nephritis.

This vascular fragility once acquired, is incurable and may in ulterior pathological circumstances give rise to new lesions (for example during delivery)

In fact the study of the different statistics published leads to a belief that any disease of the mother during pregnancy may cause auditory embryopathy or foetopathy whether it be metabolic or humoral vascular or endocrine.

With this in mind dysgravidia with threatened miscarriage, repeated haemorrhages during the greater part of the pregnancy must be admitted

The facts already known about auricular embryopathies and foetopathies are incomplete and fragmentary Alongside well-established perfectly studied facts there are many unknowns.

In fact most inquiries made into this subject up to now have been retrospective working backwards from the aural lesion they have made proof or suspicion of a causal lesion possible but not an appreciation of the exact risk

Only systematic prospective studies starting from the pathological element during pregnancy will in future allow for an evaluation of the nature and above all the extent of these risks for the baby yet to be born In other words, there must be strict surveillance of pregnancies that may be considered high risk and systematic auditory examination of all these children immediately after birth in order to draw valid conclusions.

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Finally it could be dangerous to extrapolate experimental results: for example a considerable number of medicaments may be teratogenous in animals in certain conditions of experimental procedure (salicylates or corticoids in particular). Their use in human therapy has shown that they are not necessarily noxious in normal conditions. Should a pregnant woman be deprived of aspirin because salicylate derivatives may be teratogenous in mice, rats or hamsters?

Experiments with animals produce hypotheses or suspicions rather than absolute certainties. Properly conducted, however they can be of great help in the selection of new medicaments. They make it possible if not to avoid with certainty at least to reduce to the minimum the therapeutic risk. It is due to the rigorousness of French legislation in this domain that France was spared the damage wrought by Thalidomide.

In practical terms, what prophylactic measures can be considered at the moment as far as embryopathy and foetopathy are concerned?

If one considers toxic causes and particularly medicamentous ones, within the limits of our present knowledge it is wisest to limit as far as possible the administration to pregnant women of medicaments whose innocuousness to the foetus has been proved by long experience.

If one considers infectious and, particularly viral causes, vaccination becomes all-important. Rubella in pregnant women represents a serious risk to the embryo which is now well known. A vaccine has recently been developed, its systematic use is still being discussed.

Although it does not seem very logical to make it compulsory for all girls of school age, since immunity as in the case of many vaccinations, disappears in time, it would desirable to devise a procedure of the following type. serological test at the time of the prenuptial examination in order to find out how many antibodies the young woman has; compulsory vaccination when immunity is found to be inadequate. In this case pregnancy must be strictly forbidden during three months since there would then be a major teratogenous risk.

Finally if the development of the pregnancy is in any way perturbed (intercurrent illness, threatened miscarriage, long-term therapy) very strict surveillance is essential at the time of delivery in order to avoid any factor likely to aggravate the situation—anoxia in particular—by affecting structures which are already fragile as Kelemen stresses.

Treatment

Although it is not logical to distinguish between malformations and lesions of the ear while studying the causes of embryopathies and foetopathies, the therapeutic problems vary nevertheless, according to the following circumstances. (i) whether the child has an apparent malformation of the outer or middle ear (ii) whether the child is deaf without apparent malformation.

There follows an indication of the principles which should guide therapy in each case, respectively

I. MALFORMATIONS OF THE EAR

These malformations may be very varied. They affect above all the outer and middle ear but also, more often than was previously thought, the inner ear

The pavilion, the external auditory canal, the ear drum, the tympanum, the chain of ossicles, the labyrinth, may all be affected in different degrees, including all the intermediate stages between minor and major aplasia and may be described in many dif

Table II *Aetiology of profound deafness in young children (190 cases)*

Genic causes (19.7%)		
Hereditary deafness	31	certain
Consanguinity	7	certain
Antenatal causes: embryopathy and foetopathy (9.5%)		
Rubella	15	10 certain 5 probable
Infection during pregnancy	[illegible]	probable
Dysgravidia (repeated haemorrhages during pregnancy)	[illegible]	probable
Neonatal causes (5.9%)		
Anoxia	5	
Nuclear icterus	3	
Prematurity	5	[illegible] with anoxia 3 without anoxia
Post-natal causes (20.9%)		
Meningitis: cerebro-spinal	[illegible]3	
tubercular	[illegible]	
viral	2	
Encephalitis	1	
Labyrinthic middle otitis	1	
Whooping cough	1	
Typhus	1	
Myoedema	1	
Toxic	3	
Various causes	5	
Causes unknown (40.[illegible])		

Prophylaxis

Because of the situation stated above, it is difficult at present to determine *preventive measures* and the most important thing to do is to decide upon the directions of future research.

Only the multiplication of clinical observations can bring to light the teratogenous or noxious effects on the ear of a *specified agent.* A national or international "central register" gathering together all this information would be desirable. *prospective* inquiries beginning with the pathological element occurring during pregnancy would contribute more to the appreciation of the risk incurred by the unborn child than retrospective surveys.

Alongside this clinical research experiments on *animals* could provide information of use in the field of both viral and toxic embryopathy and *foetopathy.* The limits must however be delineated.

The permeability of the placenta varies considerably between one species and another and between an animal species and human beings. It may also vary in certain circumstances (metabolic modifications, for example).

The noxious effect of a given agent may be evident in one animal, non-existent in another species, even if the permeability of the placenta is similar in both cases.

Table III *Overall table (290 cases)*

Genic causes	0
Acquired causes	
Antenatal (embryopathy and foetopathy)	10.6
Neonatal	12
Post natal	19
Unknown causes	36

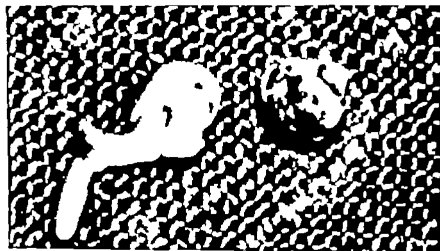
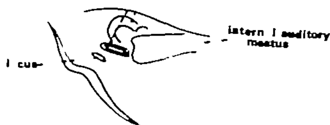


Fig 8 Malformation of incus.





Figs. 9–10 Major malformation of outer ear Radiography

ferent ways, descriptions based either on anatomopathology or on the type of deafness.

The arrival of a baby with a malformation of the ear is a *real family* tragedy and only the proposal of a precise therapeutic solution will ease the parents minds they will not be satisfied by a succession of examinations. It is above all necessary to give them precise information not only about the exact nature of the lesions, but also about the therapeutic timetable They must be reassured but they must also be told the truth without false promises. Much experience is necessary to make them agree to wait for a therapeutic answer in the distant future

There follows a few observations bearing these facts in mind.

A. The first principle of diagnosis

It is not possible to undertake a functional operation until the functional value of the ear is completely assessed.

Audiometric diagnosis is not simple it is generally submitted that a graph of free field conduction is not enough it gives only an overall picture of hearing and there may be surprises in store Before operating the otologist must know the curves of aerial and osseous conduction for each ear such a thing is not possible until the child is at least seven years old especially if only one ear is affected and it is necessary to use a technique of eliminating the hearing of the other ear The aim is to obtain accurate graphs.

Associated malformations of the outer and middle ear cause transmission deafness with an average loss of 60 dB over all frequencies it should not be forgotten that there is an audiometric and anatomopathological connection Two possibilities are described, with a clinical example (*a*) phantom curve (*b*) concomitant disorder of the inner ear

(a) Phantom curve

There are two types of phantom curve

The first type corresponds to a double phantom of the aerial and osseous curves. The ear is completely dead. The presence of total cophosis obviously affects treatment whether it is cophosis with normal contralateral ear or cophosis with disorder of the contralateral middle ear

The second type is that of the phantom of osseous conduction Deafness is not one of transmission but one of perception. This circumstance occurs as often in cases of major aplasia as in cases of supposed minor aplasia.

In all cases only vocal audiometry makes it possible to distinguish these different forms.

(b) Concomitant disorder of the inner ear

The two preceding examples have already involved the inner ear This might be suspected from radiograms, but the membranous cochlea is often isolated without any macroscopically apparent lesion When the difference of impedance is too small surgery is not the answer and a substitute method must be adopted.

Whatever the result of the audiometric examination it should be remembered that it must take its place alongside other factors. hereditary antecedents collateral and personal search for associated malformation of the cervical column the eye examination of the vestibule indicating by electrical perturbations a disorder of the membranous vestibule radiological examination simple radiogram and tomography neuropsychiatric examination giving intelligence quotient psychomotor retardation

Progress in speech is particularly interesting in cases of bilateral malformations The complete list makes it possible to ascertain the real cause or causes of retardation or lack of speech.

B. Therapy depends on whether the disorder is unilateral or bilateral

(a). Unilateral malformations

Minor malformation adolescence must be reached before surgery is performed.

Care is necessary since an abnormality of

necessary The major risk is the facial nerve. This argument has been stressed by certain authors who oppose surgery in the case of unilateral malformations. Others dispute the validity of operating to create a fenestra which might result in vertigo and difficulties when swimming.

The therapeutic calendar involves two dates, that of the functional stage in relation to the aesthetic stage. If an aesthetic stage is provided for care should be taken that the neo-pavillion is correctly placed in relation to the future external auditory canal. If the aesthetic stage is replaced by putting in place a prosthesis there is no problem.

The calendar specifies, above all, the date of the functional stage; it is not possible to operate until the child is seven for clinical and audiometric reasons as well as for surgical (the external auditory canal must be large).

It is advisable to judge the usefulness of the operation, when the controlateral ear is normal, according to the results. return to real binaural hearing is rare insistence on this fanciful idea must be abandoned, a persistent loss exists, but it is less than 30 dB the ear is then functional. A difference of impedance equal to and if possible inferior to 30 dB is the limit of tolerability If the otologist is sufficiently sure of the results, surgery is perfectly justified.

However the decision to operate is always influenced by psychological or social problems which are never negligible.

(b) Bilateral malformation

The rule about the age of seven is just as important in this case. Before seven, the child is equipped for osseous conduction and entrusted to a speech therapist so that he learns to speak in a suitable way After seven, functional surgery must be attempted, whether there are major or minor malformations, starting with the ear that is least deaf

Even partial closure of the difference of impedance is always helpful in these cases, it makes it possible to take away the apparatus from the ear

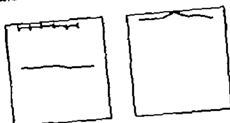


Fig. 13 Major malformation of outer ear Audiogram.



Fig 13 bis. Major malformation of middle and inner ear Audiogram.

The results of operations on malformations of the ear depend a great deal on the skill and the experience of the surgeon, but they are closely linked with the accuracy of the prescription of treatment.

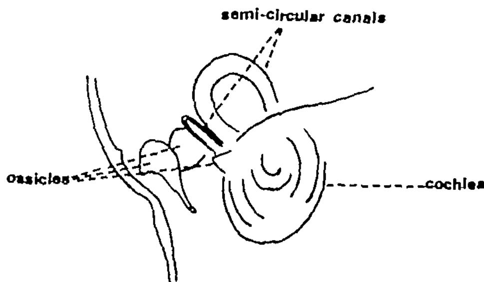
II. PERCEPTIVE DEAFNESS WITHOUT APPARENT MALFORMATION

Without wishing to undertake a study of the treatment of deaf children here, it is useful to define the present tendencies in the education of young deaf children.

Scientific publications insist on the need to begin specialized education as early as possible, when the brain is at the stage of greatest development, so that the child learns to speak as normally as possible.

This presupposes, of course, that a diagnosis of deafness can be reached with certitude within the first few years: it is not our concern at present to discuss this very important aspect of the problem. It is necessary only to remember that the audiologist has the necessary means, whether he uses classic "acumetric" methods (squeaking toys,





Figs. II 1 Major malformation of outer ear Radiography

the facial nerve may put it in a vulnerable position There are various types of lesion

(*a*) blockage mesenchymatous remains or strips stapedo-vestibular ankylosis, blockage of the chain in two places.

(*b*) interruption absence of the malleus or the malleus reduced to just the manubrium malformation of the incus malformation of the stapes no ossicles.

(*c*) absence of fenestrae

Major malformation there are two aspects to be treated aesthetic and funtional

Aesthetic aspect Fashion plays an important part it has minimised the difference between the sexes. Attitude may depend on the results obtained. Is nothing at all preferable to a semblance of an ear? The question cannot be answered. A prosthesis gives excellent results as far as appearances are concerned but the patient sometimes prefers to keep his own ear even if it is ugly The family and especially the parents, also have their own opinion upon which they often insist.

Functional aspect The otologist has two objectives to put in place a vibrating surface at the end of a new external auditory canal and to re make the chain of ossicles after a complete examination of the tympanum.

Two or even three operations are sometime

necessary The major risk is the facial nerve. This argument has been stressed by certain authors who oppose surgery in the case of unilateral malformations. Others dispute the validity of operating to create a fenestra which might result in vertigo and difficulties when swimming.

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screening test) which are still of value to a trained examiner or the excellent objective method of electro-encephalographic audiometry

It is interesting to analyse the solutions proposed for this early education. Before the child begins normal schooling, two periods can be defined schematically The first is from birth until the child is from two-and-a half to three years old, and the second from two-and-a half to three up to five or six, the age at which real schooling starts. The role of the family is fundamental in the education of a deaf child whatever its age at the time of diagnosis, but the first period is the one when the influence of the family is predominant This period is often called *mother and child education* The second period is that of the *nursery school*

A. Mother and-child education

Although it is an informal education no detail should be neglected. The meeting between the speech therapist and the mother and child should occur in a place that reminds the child of a normal home. The child is in natural surroundings, he can play have his meals, move about his mother watches him while going about her daily occupations. It is in these circumstances that the therapist comes in Meals and games are often chosen by the parents, who need specific much more than general guidance The behaviour of each child is closely examined on different occasions. It is assessed according to certain criteria and carefully noted However this analysis is complemented by a study of the attitude of the parents towards the child, in particular that of the mother This wonderful opportunity of observing both mother and child chould be used for one two or three days. Such close observation the assessment and recording of the facts observed are indispensable if valid advice is to be given to the parents. Furthermore this information must be in such a form that it may be used directly by all those who come into contact with or are in charge of the deaf child. This really scientific study of *the child* should make it possible with a certain margin of error to discover what kind of education will suit him best.

It is *not until after* this extremely long phase *of observation that* education begins. The speech therapist works in different fields, she *tries* to develop the attention and the visual *memory* to form judgement by classification exercises for example. She tries to draw the *child s attention* and interest towards the movements of the lips, *first of all by* breathing exercises, then by association with objects or situations. She develops the child s capacity for imitating movements of the body limbs, mouth tongue and lips. *All these exercises* are presented as games. The whole auditory education including the important part played by the initiation to rhythm is covered in these first game-exercises.

The frequency of sessions varies and depends on the distance and the availability The child stays in a specialized centre either for a day or for several days, with intervals of from fifteen days to a month

This is the way that mother and-child education is conducted at the Institut d Audiophonologie in Lyon. This solution can certainly be used in a large number of cases, but not in those where both the mother and father are at work There ought to be day-nurseries where the child could play under the supervision and with the help of a speech therapist the child would be taken there in the morning and taken back by his parents in the evening We find this solution less satisfactory than the first.

B. Nursery-schools for deaf children

When the child is older he may go to a nursery school If possible he is taken there, he plays and works for the morning or the whole day going home to his family every day Nursery-schools for the deaf have become quite customary Putting the child into a specialized centre for the deaf involves, in objective terms, the same dangers as boarding-

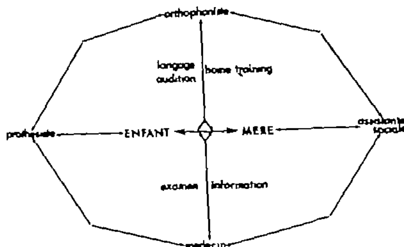


Fig. 14 Diagram of early identification and rehabilitation of young deaf children.

school. Ideally the child should enjoy the family atmosphere every day so that he remains in contact with his parents and brothers and sisters. A specialized nursery-school for the deaf is only possible in a large town for families in rural areas and small towns the problems are complex and the best use must be made of mother-and-child education in the centre up to nursery-school age, between periods of specialized education the child goes to an ordinary nursery-school.

It is the contact between the therapist and the child and his mother that makes it possible to fit into the most ordinary of daily acts the introduction of the child to language to human communication. Hearing aids are included in this rehabilitation. This is possible since modern audiometric methods are improving all the time and the auditory loss can be diagnosed with great accuracy When the exact auditory loss is known and informal and, in particular auditory education has begun a hearing aid can be considered as early as possible according to the preceding conditions.

It must not be pretended that the education of a deaf child depends on early education alone. There are fundamental factors over which the doctor and the therapist have no control. The intelligence quotient is very important. The higher it is, the easier is the child's education. The profundity of deafness is also, in our opinion very important. The profoundly deaf child is one whose handicap is greatest to overcome. This child benefits greatly from early education, even from early use of a hearing aid, but the result will not be as good as when there is only partial deafness which responds better to aids. The best results are still achieved in cases of partial deafness. In these circumstances early education gives the most spectacular results, in fact the child may be able to go to an ordinary school and follow under medical and therapeutic control, a perfectly normal education.

Summary

As far as the aetiology of deafness in children is concerned, the pathology of pregnancy is a field which is still relatively unexplored although it is of capital importance when considering the prophylaxis of the problem.

Embryological data shows that although the

ear is anatomically constituted by the end of the third month of pregnancy histological modifications continue until the sixth month and the histochemical evolution perhaps continues even well beyond that

It is difficult therefore to consider separately auricular embryopathies and foetopathies, especially since the same pathological agent may intervene at either the embryo stage or the foetal stage.

Different factors are liable to damage the ear or to perturb its development before birth

Of the *infectious causes* the rubella virus holds first place its form of action the different clinical aspects of the rubella embryopathy are now well known

Other viral or microbic agents such as measles mumps, influenza shingles pneumococci, streptococci etc that is, they are probable causes which cannot yet be definitely confirmed

In the case of *gravidic parasitosis* the danger toxoplasma represents to the foetus must be stressed. The effect on the ear of congenital toxoplasmosis has been described only recently but it is likely that this aspect will become very important in future

Ionising radiation has for a long time been suspected of having a teratological effect but little has been published on the elective affections of the ear

The study of embryopathies and foetopathies of toxic origin—whether the noxious substance be exogenic, medicamentous in particular or endogenous—has recently become of great and immediate interest as a result of the dramatic consequences of the use of Thalidomide.

Because of the continued presence of many unknown factors in this field, it is difficult to provide general rules for prophylaxis at present the centralization of observations and experiments with animals will undoubtedly lead to progress in the future.

In practice the preventive measures at the disposal of the clinician are caution in the administration of medicamentous treatment to pregnant women vaccination against rubella and close supervision of labour to prevent the possible aggravation of lesions by anoxia.

All too often it is possible only to treat a child already malformed or suffering from deafness to a greater or lesser extent. The fundamental principles then guiding the otologist are early education of the young deaf child at first within the family and later in a specialized nursery school information indicating whether the subject is unilaterally or bilaterally affected in the case of auricular malformation

Zusammenfassung

Der Einfluss verschiedener Krankheiten während der Schwangerschaft auf angeborene Schwerhörigkeit oder Gehörlosigkeit bei Kindern ist ein noch wenig erforschtes Kapitel, obwohl es von prophylaktischem Standpunkt aus gesehen ungeheuer wichtig ist

Die embryonale Entwicklungsgeschichte zeigt dass die anatomischen Ansätze des Gehörorganes am Ende des dritten Schwangerschaftsmonats ausgebildet sind dass sich aber die histologischen Veränderungen bis zum sechsten Schwangerschaftsmonat hinausziehen und die histo-chemischen Prozesse noch länger bis zu ihrer Vollendung brauchen

Es ist daher schwierig, genau zwischen Embryopathien und Foetopathien zu unterscheiden zumal die verschiedenen Krankheitserreger während beider Entwicklungsperioden

wirken können. Verschiedene Faktoren können das Gehörorgan während seiner Entwicklung zerstören oder hemmen.

Viren und Bakterien

Der Rotel-Virus (rubeolis) nimmt eine Vorzugsstellung ein. Seine Wirkungsweise, die verschiedenen klinischen Aspekte und die verursachten Embryopathien sind jetzt gut bekannt. Andere Viren und Bakterien sind genannt worden: Virus der Masern, des Mumps, der Grippe und auch Pneumococcus, Streptococcus ... Es handelt sich bei den Letzteren um mögliche aber nicht bestätigte Ursachen.

Parasiten

Es soll unterstrichen werden, dass der Krankheitserreger der Toxoplasmose Einfluss auf die Entwicklung des Gehörorganes nimmt. Die angeborene Toxoplasmose des Gehörorganes ist bisher wenig beschrieben worden, aber es scheint, dass diese Ursache in Zukunft naher in Betracht zu ziehen ist.

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Die Vermutung, dass ionisierende Strahlen Ursache von Missbildungen am Gehörorgan sein können, ist schon lange aufgestellt worden. Man findet aber wenige Dokumente in der Literatur, dass diese isolierten Schäden am Gehörorgan verursachen.

Endogene und Exogene Vergiftungen

Das Studium dieses Kapitels hat nach den traurigen Ereignissen infolge Gebrauchs von Kontergan bei schwangeren Frauen an Wichtigkeit gewonnen. Neben den exogenen Ursachen, bei denen die Medikamente eine Sonderstellung einnehmen, können auch endogene Vergiftungsursachen erwogen werden.

Leider bestehen auf diesem Gebiete noch viele Unklarheiten und man kann kaum prophylaktische Ratschläge geben. Vielleicht ermöglicht eine zentrale Auswertung der klinischen Fälle und der Tierexperimente baldige Fortschritte. Bis auf weiteres kann der Kliniker nur Vorsicht beim Gebrauch von Medikamenten bei Schwangeren walten lassen, Impfung gegen Röteln zum gegebenen Zeitpunkt vornehmen und die Ursachen des Sauerstoffmangels während der Entbindung bekämpfen, um eventuell schon bestehende Schäden nicht noch zu erschweren.

Leider steht der Kliniker aber noch oft den Schäden am Gehörorgan auf dem therapeutischen Stadium gegenüber und es handelt sich darum, sich mit einem missgebildeten, mehr oder weniger schwerhörigen Kind zu befassen. Einige Grundsätze leiten dann den Spezialisten. Frühzeitige Erziehung des Schwerhörigen — erst im Familienkreise und dann in Sondereinrichtungen, Wahl unter verschiedenen Behandlungsmethoden je nachdem ob die Schwerhörigkeit nur eine oder beide Seiten betrifft.

Resumé

La pathologie de la grossesse représente un chapitre encore assez mal exploré dans l'étiologie des surdités de l'enfant et cependant d'une importance capitale, si l'on considère l'aspect prophylactique du problème.

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dant la période embryologique que pendant la période foetale

Différents facteurs sont susceptibles de léser l'oreille ou d'en perturber le développement pendant la vie intra utérine

Parmi les *causes infectieuses* le virus de la rubéole tient la première place ses modalités d'action, les différents aspects cliniques de l'embryopathie rubéolique sont maintenant bien connus.

D'autres agents viraux ou microbiens sont également incriminés tels ceux de la rougeole, des *oreillons*, de la grippe du zona ou encore pneumocoque streptocoque etc. il s'agit pour l'*instant* de causes probables que l'on ne peut affirmer avec certitude

Il faut souligner dans le cas des *parasitoses gravidiques* le risque que fait courir au foetus le toxoplasme. L'atteinte auriculaire de la *toxoplasmose* congénitale a été décrite depuis peu mais il semble que cette étiologie soit appelée à prendre une place importante

Les *radiations ionisantes* sont depuis long temps soupçonnées d'une action tératologique mais la littérature reste assez pauvre en ce qui concerne les atteintes électives de l'oreille

Quant aux embryopathies ou foetopathies d'*origine toxique* — la substance nocive pouvant être exogène, médicamenteuse surtout, ou endogène — leur étude a pris une actualité et une importance majeure à la suite des dramatiques conséquences de l'utilisation de la Thalidomide.

Du *fait des nombreuses inconnues* persistant à l'heure actuelle dans ce domaine il est difficile de définir les règles prophylactiques générales centralisation des observations, expérimentations chez l'animal permettront, sans nul doute des progrès futurs.

En pratique, prudence dans le maniement des thérapeutiques médicamenteuses chez la femme enceinte vaccination contre la rubéole, surveillance étroite de l'accouchement pour éviter l'aggravation des lésions par une éventuelle anoxie représentent les mesures préventives essentielles à la disposition du clinicien.

Trop souvent encore c'est au stade thérapeutique qu'il faut agir devant un enfant malformé ou porteur d'une surdité plus ou moins sévère Quelques principes fondamentaux doivent alors guider l'otologiste éducation précoce du jeune enfant sourd dans sa famille d'abord, puis dans un jardin d'enfants spécialisé indications centrées sur l'uni- ou la bi-latéralité de l'atteinte dans le cas de malformations auriculaires.

Bibliography

We have limited ourselves to a few recent references. For publications earlier than 1965 the complete bibliographies of the following works may be consulted

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dant la période embryologique que pendant la période foetale

Différents facteurs sont susceptibles de léser l oreille ou d en perturber le développement pendant la vie intra utérine.

Parmi les *causes infectieuses* le virus de la rubéole tient la première place ses modalités d action les différents aspects cliniques de l embryopathie rubéolique sont maintenant bien connus.

D autres agents viraux ou microbiens sont également incriminés tels ceux de la rougeole des oreillons, de la grippe du zona ou encore pneumocoque, streptocoque etc il s agit pour l instant de causes probables que l on ne peut affirmer avec certitude.

Il faut souligner dans le cas des *parasitoses gravidiques* le risque que fait courir au foetus le toxoplasme. L atteinte auriculaire de la toxoplasmose congénitale a été décrite depuis peu mais il semble que cette étiologie soit appelée à prendre une place importante

Les radiations ionisantes sont depuis longtemps soupçonnées d une action tératologique mais la littérature reste assez pauvre en ce qui concerne les atteintes électives des l oreille.

Quant aux embryopathies ou foetopathies d *origine toxique* — la substance nocive pouvant être exogène, médicamenteuse surtout ou endogène — leur étude a pris une actualité et une importance majeure à la suite des dramatiques conséquences de l utilisation de la Thalidomide

Du fait des nombreuses inconnues persistant à l heure actuelle dans ce domaine il est difficile de définir les règles prophylactiques générales centralisation des observations, expérimentations chez l animal permettront sans nul doute des progrès futurs.

En pratique prudence dans le maniement des thérapeutiques médicamenteuses chez la femme enceinte vaccination contre la rubéole surveillance étroite de l accouchement pour éviter l aggravation des lésions par une éventuelle anoxie représentent les mesures préventives essentielles à la disposition du clinicien

Trop souvent encore c est au stade thérapeutique qu il faut agir devant un enfant malformé ou porteur d une surdité plus ou moins sévère. Quelques principes fondamentaux doivent alors guider l otologiste éducation précoce du jeune enfant sourd dans sa famille d abord, puis dans un jardin d enfants spécialisé, indications centrées sur l uni- ou la bi latéralité de l atteinte dans le cas de malformations auriculaires.

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Introduction

The study of the transport of ions and molecules across Reissner's membrane is important for understanding the origin and circulation of the labyrinthine fluids. The first evidence of transport of ions across this membrane was obtained by Rauch, Köstlin, Schneider & Schindler (1963) who injected ^{24}Na and ^{42}K compounds in the perilymph and measured the concentration of labelled ions in both endolymph and perilymph. ^{42}K and ^{24}Na crossed Reissner's membrane from perilymph to endolymph, but the concentration of ^{42}K in the endolymph was about five to eight times greater than Na. When injecting enzyme blocks, either iodo acetate, sodium cyanide or strophanthin G into the perilymphatic space prior to injecting ^{42}K and Na, the radioactivity of the endolymph was reduced by approximately two-thirds the amount previously measured. By means of these biochemical methods they concluded that Reissner's membrane actively transports ions from perilymph toward endolymph.

Ilberg (1968) has provided morphological evidence of a transport mechanism across Reissner's membrane. He injected a colloidal suspension of thorium dioxide into the perilymphatic or endolymphatic space as a marker. This substance crossed Reissner's membrane in both directions by a combination of diffusion and active transport mechanisms. He also reported when remaining in the cochlear duct for more than 30 minutes, thorium dioxide produced toxic changes in the cells of Reissner's membrane.

Several aspects of Ilberg's important work led us to reinvestigate the transport mechanism across Reissner's membrane. Firstly the possibility arose that the toxicity of thorium dioxide may have altered the transport of macromolecules across Reissner's membrane. Hence, it seems advisable to study this process by means of a different substance, namely ferritin, which is more biological and less toxic electron-opaque tracer. A second aspect we considered was that injection of a substance in either space may rupture Reissner's membrane resulting in a mixture of endolymph and perilymph and thereby contaminating both fluids with the tracer. When this occurs the amplitude of the cochlear potentials decreases significantly (Davis et al., 1955, Lawrence et al., 1961). The endocochlear potential (EP) provides an excellent indicator of the Reissner's membrane integrity. It can be monitored by means of a microelectrode inserted in the endolymph and in absence of acoustic stimulation its value is about 80 millivolts positive relative to the perilymph and the tissue fluids generally. A third point is that fixation of the tissue by immersion is inadequate because all the cochlear structures are surrounded by bone with only narrow channels for diffusion of the fixative. Thus a fixation in vivo by perfusion of the fixative through scala tympani and with an outlet in scala vestibuli or vice versa is necessary.

For the reasons stated above, we investigated the transport of macromolecules across Reissner's membrane using ferritin as electron-opaque tracer. The integrity of Reissner's membrane was monitored through EP recording during and after injection of ferritin in the labyrinthine spaces. Adequate fixation of Reissner's membrane was accomplished by intracochlear perfusion of the fixative *in vivo*.

Our data indicate that transport of ferritin takes place across Reissner's membrane from the perilymph toward the endolymph but not in the opposite direction.

List of Abbreviations

BL, Basal lamina
D Desmosome
DB Dense body
End C, Endolymphatic cell
Es, Endolymphatic space
Gc Golgi complex
ger Granular endoplasmic reticulum
Is Intercellular space
m Mitochondria
MVB Multivesicular body
N Nucleus
Per C, Perilymphatic cell
Ps, Perilymphatic space
ZA, Zonula adherens
ZO Zonula occludens

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Materials and Methods

The experiments were carried out in a series of 22 healthy adult cats of which 5 serve as controls. Each animal was anesthetized with intraperitoneal pentobarbital sodium (0 6 ml per kg of body weight) Tracheotomy was per formed and the cat s head was fixed rigidly in a restraining device until no respiratory displacements could be observed under high amplification of a dissecting microscope A retroauricular approach to the middle ear was carried out exposing the round window niche.

Technique for endolymphatic injection of ferritin

Two glass pipettes were mounted side by side on a micromanipulator One whose diameter at the tip was about 8 μ contained an estimated volume of 0 10 to 0.33 μl of ferritin solution This pipette was connected by a polyethylene tube to a hydraulic microdrive. The other pipette whose tip diameter was about 2 μ was filled with 3 M NaCl or 0 1 M KCl

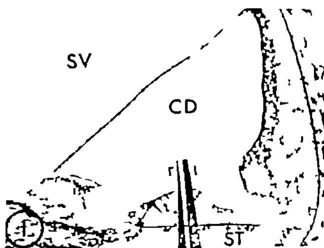


Fig 1 Photomicrograph of the cochlear duct of the cat showing an outlin f two pipettes, one for re cording endocochlear potential ( ), and the other for injection of ferritin in the cochlear duct (*i*). The position of the pipettes in the organ of Corti and depth of penetration in he cochlear duct represent only an estimate. *SI* Scala estibul *CD* Cochlear duct, *ST* Scala tympani. 97

it was used for simultaneous recording of the endocochlear potential (EP).

The membrane covering the round window was removed, thus permitting adequate visual observation of the basilar membrane. With the aid of a high power dissecting microscope the basilar membrane was impaled at the level of the Claudius cells and about 3 mm from the base of the cochlea. The pipettes were advanced slowly until the recording mic ropipette recorded a positive EP of about 80 mV indicating that the tips of the pipettes were in the cochlear duct (Fig. 1) The endocochlear potential was recorded by an elec trometer (E–H Research Laboratories, Model 250) and registered on an Offner Dynograph. The ferritin solution was then slowly injected throughout a 1 to 2 minute interval. After completion of the injection an approximately equal volume of endolymph was withdrawn The elapsed time between completion of fer ritin injection and intravital fixation of the cochlea was 10 20 30 60 or 120 min during which time the EP was recorded during the first 5 min following ferritin injection.

Technique for perilymphatic injection of ferritin

A glass pipette, 150–250 μ inside diameter at the tip was mounted on a micromanipula tor and connected by means of a polyethylene tube to a syringe containing 2 cc of ferritin solution the syringe was placed on a Harvard infusion withdrawal pump The pipette was filled to the tip with ferritin solution, thus eliminating all bubbles. The pipette was then gently introduced through the round window membrane into the scala tympani The stapes was removed and the ferritin solution was in jected at a rate of 0.68 ml per minute for approximately 3 min After an interval of

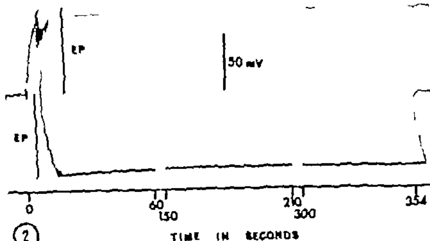


Fig 2 Cat Fe-19 Record of negative potential (EP^- – 80 mV) within the organ of Corti and positive endocochlear potential (EP^+ 76 mV) within the cochlear duct. After penetrating the cochlear duct, EP^+ exhibited slow progressive decay (6 mV) reaching a steady state in one minute. Injection of ferritin into scala media (time of injection indicated by two arrow heads) apparently was not associated with changes in the course of EP^+ Calibration marker – 50 mV

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Preparation of ferritin stock solutions

Horse spleen cadmium-free ferritin (1 gm in 10 ml) was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio). Commercial cadmium-free" ferritin contains traces of the cadmium sulphate used for crystallization of the protein (Eybl & Ryser 1964). In order to remove these toxic traces, the ferritin solution was dialysed at 2 C for 72 hours against three changes of 3.7% disodium dihydrogen versenate (EDTA) in 0.15 M phosphate buffer at pH 7.0, followed by additional dialysis for three days against three changes of Gey s balanced salt solution at 2 C (Farquhar & Palade, 1961). The ferritin solution was then tested for the presence of cadmium following the method of Granick (1942).

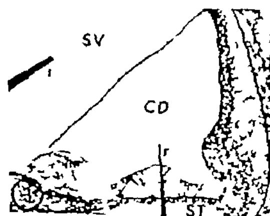


Fig 3 Photomicrograph of the cochlear duct of the cat showing the position of the micropipette for recording endocochlear potential (*r*). The micropipette for injection of *ferritin* in the perilymphatic space (*f*) was introduced through the oval window membrane into the vestibule. *SV* Scala vestibuli, *CD* Cochlear duct; *ST* Scala tympani. 97

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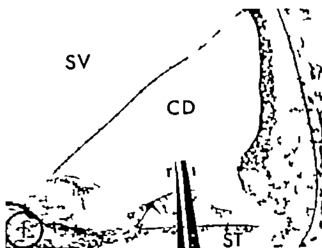


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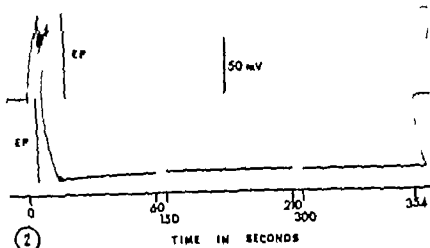


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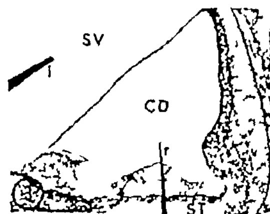


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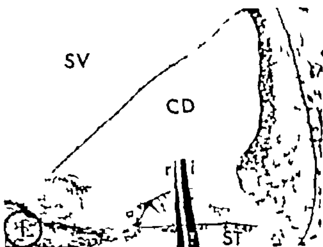


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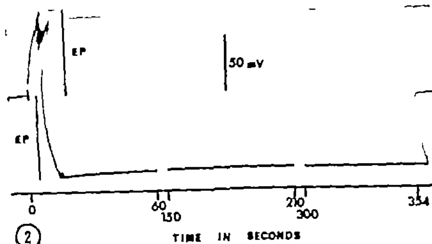


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Preparation of ferritin stock solutions

Horse spleen cadmium-free ferritin (1 gm in 10 ml) was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio). Commercial cadmium-free" ferritin contains traces of the cadmium sulphate used for crystallization of the protein (Eybl & Ryser 1964). In order to remove these toxic traces, the ferritin solution was dialysed at 2 C for 72 hours against three changes of 3.7% disodium dihydrogen versenate (EDTA) in 0.15 M phosphate buffer at pH 7.0, followed by additional dialysis for three days against three changes of Gey's balanced salt solution at 2 C (Farquhar & Palade 1961). The ferritin solution was then tested for the presence of cadmium following the method of Granick (1942).

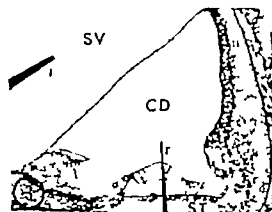


Fig. 3 Photomicrograph of the cochlear duct of the cat showing the position of the micropipette for recording endocochlear potential (*r*). The micropipette for injection of ferritin in the perilymphatic space (*i*) was introduced through the oval window membrane into the vestibule. *SV* Scala vestibuli; *CD* Cochlear duct; *ST* Scala tympani. 97

Materials and Methods

The experiments were carried out in a series of 22 healthy adult cats of which 5 serve as controls. Each animal was anesthetized with intraperitoneal pentobarbital sodium (0 6 ml per kg of body weight). Tracheotomy was per formed and the cat s head was fixed rigidly in a restraining device until no respiratory displacements could be observed under high amplification of a dissecting microscope A retroauricular approach to the middle ear was carried out exposing the round window niche

Technique for endolymphatic injection of ferritin

Two glass pipettes were mounted side by side on a micromanipulator One whose diameter at the tip was about 8 μ contained an estimated volume of 0 10 to 0.33 μl of ferritin solution. This pipette was connected by a polyethylene tube to a hydraulic microdrive The other pipette whose tip diameter was about 2 μ was filled with 3 M NaCl or 0 1 M KCl

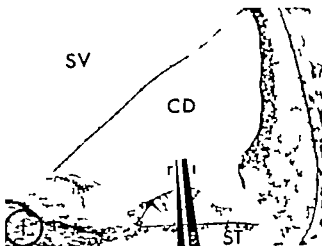


Fig 1 Photomicrograph of the coch r duct of th cat showing an outline f tw p pet one for recording endocochlear pote al ( ), and the other for injection of ferritin in the ochlear duct (*i*). The position of the pipettes in the organ of Corti and depth of penetration n he cochlear duct represent only an estimate. *SI* Scala vestibuli *CD* Cochlear duct *ST* Scala tympani. 97

it was used for simultaneous recording of the endocochlear potential (EP)

The membrane covering the round window was removed thus permitting adequate visual observation of the basilar membrane. With the aid of a high power dissecting microscope the basilar membrane was impaled at the level of the Claudius cells and about 3 mm from the base of the cochlea. The pipettes were advanced slowly until the recording micropipette recorded a positive EP of about 80 mV indicating that the tips of the pipettes were in the cochlear duct (Fig. 1) The endocochlear potential was recorded by an electrometer (E–H Research Laboratories, Model 250) and registered on an Offner Dynograph. The ferritin solution was then slowly injected throughout a 1 to 2 minute interval. After completion of the injection an approximately equal volume of endolymph was withdrawn. The elapsed time between completion of ferritin injection and intravital fixation of the cochlea was 10 20 30 60 or 120 min during which time the EP was recorded during the first 5 min following ferritin injection

Technique for perilymphatic injection of ferritin

A glass pipette, 150–250 μ inside diameter at the tip, was mounted on a micromanipulator and connected by means of a polyethylene tube to a syringe containing 2 cc of ferritin solution the syringe was placed on a Harvard infusion/withdrawal pump. The pipette was filled to the tip with ferritin solution thus liminating all bubbles. The pipette was then gently introduced through the round window membrane into the scala tympani. The stapes was removed and the ferritin solution was injected at a rate of 0.68 ml per minute for approximately 3 min After an interval of

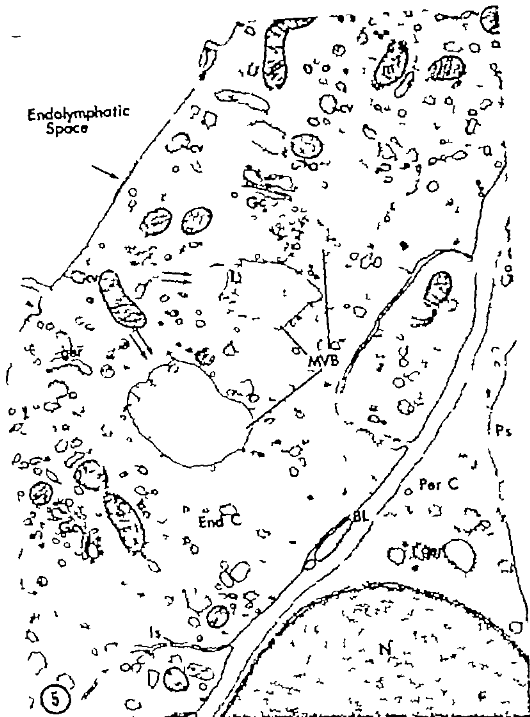


Fig. 5. Low power electron micrograph of Reissner membrane 30 min after injection of ferritin in the endolymph. Ferritin molecules are seen adhering to localized area of filamentous coating of the outer surface of the membrane (*arrow*). The tracer is also visible in vesicular invagination (*arrow head*) of the free surface of the endolymphatic cell and in coated vesicles (*cv*). The latter are accumulated near the free surface of the cell. Three multivesicular bodies containing ferritin molecules are seen in different stages of development. These bodies are surrounded by numerous round vesicles. A localized dense material is seen in the cytoplasmic side of the membrane of two multivesicular bodies (*double arrow*). This material is projected for a variable distance into the cytoplasm. No ferritin molecules were found in the intercellular space, basal lamina or in the perilymphatic cell. 3 085

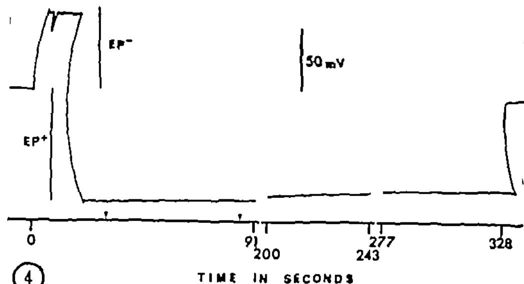


Fig 4 Cat Fe 7 Record of the endocochlear potentials after injection of ferritin in the perilymph. The negative potential (EP^-) within the organ of Corti was 60 mV and the positive endocochlear potential (EP) was 90 mV EP exhibited slow progressive decay of about 14 mV in 5 min Time of injection of ferritin is indicated by two arrow heads. Calibration marker = 50 mV

The ferritin solution was sterilized by passing it through a 0 45 μ mesh Millipore filter The filtrate was received in covered sterile ampules which were immediately refrigerated. The concentration of ferritin was estimated to be approximately 100–125 mg/ml in the sterile solution

Technique for intravital fixation of the cochlea[1] and subsequent treatment

The intravital fixation of the cochlea and subsequent treatment were done identically in the experimental animals and controls. A glass pipette of 150–250 μ inside diameter at the tip was mounted on a micromanipulator The pipette was connected through a polyethylene tube to a syringe containing 30 0 cc of 2.67% solution of osmium tetroxide in s-Collidine buffer at pH 7 4 (Bennett & Luft 1959). To maintain the temperature of the fixative at about 4 C during perfusion of the cochlea the syringe was mounted in a copper container filled with ice The syringe and the copper container were placed on a Harvard infusion/withdrawal pump this instrumentation being ready for immediate use after completing ferritin injection

After removal of the pipettes used for the endolymphatic injection of ferritin and EP recording, the oval window was exposed and both crurae of the stapes were removed. The pipette used for perfusion was first completely filled with fixative to eliminate all bubbles. Then it was introduced gently through the footplate of the stapes into the vestibule and the pump was activated The fixative was flowed through the scala vestibuli scala tympani and out through the round window being gently sucked from the middle ear cavity Direct observation of both round and oval windows showed that most of the fixative flowed out of the round window although some fluid seepage between the pipette and the borders of the perforation in the footplate of the stapes was noticed. The perfusion rate was 1 23 ml per minute hence the total perfusion time was about 24–25 min

In the case of perilymphatic injection of

[1] This technique was published in a preliminary form in Hinojosa R & Rodriguez Echandia, E. L. 1966 The fine structure of the stria vascularis of the cat inner ear *Am r J Anat 118*, 631

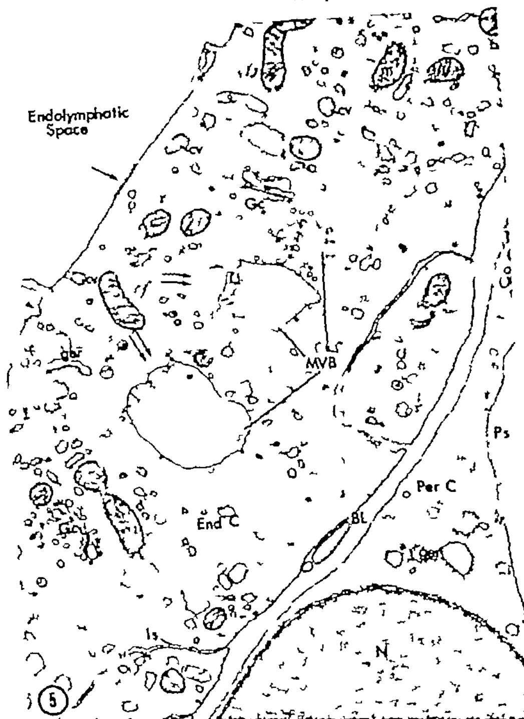


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ferritin the pipette and polyethylene tube used for injection of the marker were kept in place but the syringe was replaced with a 30.0 cc syringe containing fixative. The fixative flowed from scala tympani into scala vestibuli and out through oval window. In some animals the flow direction was reversed.

Upon completion of perfusion the anesthetized animal was decapitated and the cochlea removed from the temporal bone. Under a dissecting microscope, the lateral wall of the otic capsule was removed rapidly with a fine chisel and the cochlea immersed in the fixative for 3 1/2 hours. After fixation the specimen was transferred to cold 70% ethanol and the cochlear was partitioned under a dissecting microscope into a number of small pieces. The specimens were dehydrated in increasing concentrations of ethanol and then embedded in Epon 812 according to Luft (1961).

Sections were obtained with an LKB Ultrotome III equipped with a diamond knife collected on copper grids covered with carbon film (Robertson, 1959) and stained with lead tartrate (Millonig, 1961) or lead citrate (Reynolds, 1963). The sections were examined and photographed in a Philips EM 300 Electron Microscope operated at 40 kV. Thick sections of about 1 μ were cut with a glass knife mounted on slides and covered with immersion oil under a coverslip. These sections were examined with phase contrast under a Zeiss Photo-microscope.

Observations

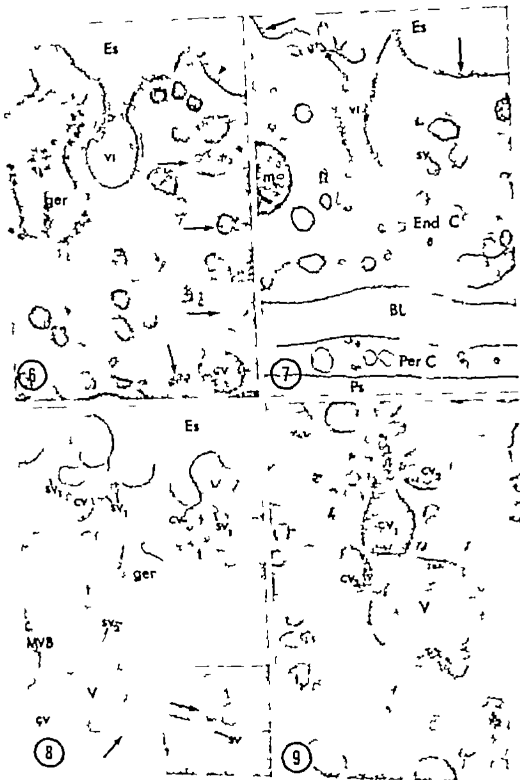
The ultrastructure of Reissner's membrane has been fully described in the literature (Hagiwara, 1963; Duvall & Rhodes, 1967; Iurato 1967; Iurato & Taidelli, 1967) thus a short summary of the most important features related to our work is here included. Reissner's membrane separates the endolymphatic from the perilymphatic space. The membrane is a thin epithelium which varies from 1.2 μ to 3.3 μ in thickness, it consists

Fig. 6. An area of an endolymphatic cell fixed 1 hour after injection of ferritin in the endolymph showing a vesicular invagination of the free surface (vi) with numerous ferritin molecules which are apparently attached to the external filamentous coating of the membrane. The cytoplasmic side of this vesicular invagination is covered with thin radially striated layer. A localized area of the plasmalemma shows an external filamentous coating to which few ferritin molecules are apparently attached (*arrow head*). Ferritin molecules are also seen in small vesicles (*arrows*) and in a coated vesicle (*cv*). 80.556.

Fig. 7. Reissner's membrane 1 hour after injection of ferritin in the endolymph showing a portion of an endolymphatic cell, basal lamina and perilymphatic cell. Ferritin molecules are apparently adherent to areas of external coating of the cell membrane (*arrow*). Numerous molecules of the tracer are seen in a channel-like tubular invagination (*t*) of the plasmalemma. A small vesicle (*v*) with a single ferritin molecule is found in the cytoplasm. No tracer molecules are found in either the basal lamina or perilymphatic cell. 44 893.

Fig. 8. A portion of an endolymphatic cell 1 hour after injection of ferritin in the endolymph. The tracer is found in small vesicles (*sv1*), coated vesicles (*cv*), tubular structures (*t*) and in other small vesicles (*sv2*) located near a vacuole (*V*) which also contains ferritin. A small vesicular profile (*arrow*) seems to be fused with or budding from the vacuole (*V*). The diameter of the small vesicles is 650-750 Å. *Inset*. This micrograph shows a small vesicular invagination (*arrow*) of the free surface of a microvillus. The cell membrane shows, at the left of the inset, a localized area of external filamentous coating in which few ferritin molecules are apparently attached. 53 35... *Inset* 82 080.

Fig. 9. An area near the free surface of an endolymphatic cell 30 min after injection of ferritin in the endolymph. A coated vesicle (*cv*) continuous with a tubular structure contains ferritin molecules. Both the coated vesicle (*cv*) and the tubular structure exhibit a continuous inner coating but notice the difference in density of their lumen. The tracer also appears in other coated vesicles (*cv*), a vacuole (*V*) and in some irregular tubular structures (*t*). Another tubular structure (*t1*) with a bulb-like dilatation also contains numerous ferritin molecules. 74 898.


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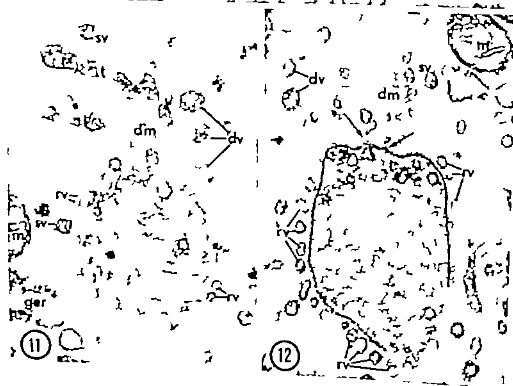
Fig 6 An area of an endolymphatic cell fixed 1 hour after injection of ferritin in the endolymph showing a vesicular invagination of the free surface (vI) with numerous ferritin molecules which are apparently attached to the external filamentous coating of the membrane. The cytoplasmic side of this vesicular invagination is covered with thin radially striated layer A localized area of the plasmalemma shows an external filamentous coating to which few ferritin molecules are apparently attached (*arrow head*). Ferritin molecules are also seen in small vesicles (*arrows*) and in a coated vesicle (*cv*). 80 256.

Fig 7 Reissner's membrane 1 hour after injection of ferritin in the endolymph showing a portion of an endolymphatic cell basal lamina, and perilymphatic cell Ferritin molecules are apparently adherent to areas of external coating of the cell membrane (*arrows*) Numerous molecules of the tracer are seen in a channel-like vesicular invagination (*I*) of the plasmalemma. A small vesicle (*sv*) with a single ferritin molecule is found in the cytoplasm. No tracer molecules are found in either the basal lamina or perilymphatic cell. 44 893

Fig 8 A portion of an endolymphatic cell 1 hour after injection of ferritin in the endolymph. The tracer is found in small vesicles (*v1*), coated vesicles (*cv*), tubular structures (*t*) and in other small vesicles (v_2) located near a vacuole (*V*) which also contains ferritin. A small vesicular profile (*arrow*) seems to be fused with or budding from the vacuole (*V*). The diameter of the small vesicles is 650–750 Å. *Inset* This micrograph shows a small vesicular invagination (*arrow*) of the free surface of a microvillus. The cell membrane shows, at the left of the inset, a localized area of external filamentous coating in which few ferritin molecules are apparently attached 53 35.. *Inset* 82 080

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of two layers of cells which can be distinguished by their shape location and cytoplasmic characteristics. A distinct basal lamina (basement membrane) is found between the two cellular layers. The cells facing the endolymphatic space referred to as endolymphatic cells, are low cuboidal and their free surface shows numerous short microvilli. Our control material also showed that the microvilli contained small uncoated vesicles which vary in number from one microvillus to another The cell membrane measures 90 Å in thickness and shows numerous vesicular invaginations from which coated vesicles are formed Both types of vesicles, small vesicles and coated vesicles, accumulate in the apical cytoplasm. Numerous multivesicular bodies, vacuoles, mitochondria granular endoplasmic reticulum and elements of the Golgi complex are found dispersed throughout the cytoplasm. Adjacent cells are attached by typical junctional complexes consisting of a zonula occludens, zonula adherens and a macula adherens or desmosome. The lower third of the lateral plasmalemma characteristically follows a meandering course Adjacent endolymphatic cells are separated by a 150 to 200 Å intercellular cleft. The perilymphatic cells, i.e those facing the perilymphatic space are thin and elongated. The relatively few organelles, contained in these cells are located mainly around the nucleus. Small uncoated vesicles are found distributed all along the cytoplasm. The margins of adjoining perilymphatic cells either abut bluntly or overlap (Fig 17) in either case there is a simple junctional complex consisting of regions of simple apposition of the unit membranes (Fig. 17) The basal lamina is a continuous layer of apparently amorphous material 300 to 500 Å in thickness it follows the small irregularities of the basal plasmalemma of the endolymphatic cells.

Endolymphatic Injection

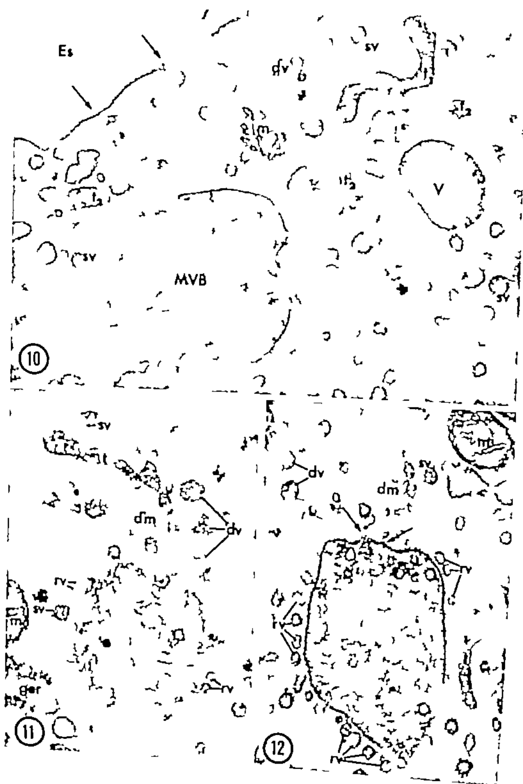
While advancing the micropipette through the organ of Corti two potentials were found. First as the recording pipette penetrated the basilar membrane into the organ of Corti, a negative potential (EP) of about 80 mV was

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Fig 10 A portion of an endolymphatic cell, 30 min after injection of ferritin in the endolymph, showing ferritin molecules closely apposed to localized areas of external coating of the free surface of the cell (*arrows*). An irregular tubular structure (*t*), with dense lumen, contains numerous ferritin molecules which are mainly accumulated in the bulb-like dilatations. The tracer also appears in other tubular structures (t_1), and in the lumen of both a vacuole (*V*) and a *multivesicular body* Notice in the last two organelles an inner filamentous coating of their limiting membrane in which numerous ferritin molecules are apparently embedded. Notice also that in the lumen of the multivesicular body the ferritin molecules tend to be distributed in a linear pattern. A dense round structure (*dr*), which apparently presents no limiting membrane, contains few ferritin molecules. The tracer also appears in dense small vesicles (*s*) of about 900 Å in diameter 58 48…

Fig 11 Multivesicular body in an endolymphatic cell two hours after injection of ferritin into the endolymph It shows accumulation of dense material (*dm*) at one pole of the body A tubular structure (*t*) and few vesicular profiles, *some of them with ferritin molecules are surrounded by dense material* (*dm*). Few small vesicles (*n*) and dense vesicles (*d*), all containing ferritin, are seen close to the multivesicular body which shows numerous ferritin molecules. A few round vesicles (*rv*) are seen near the multivesicular body 60 534

Fig 12 Multivesicular body of an endolymphatic cell, 30 min after injection of ferritin in the endolymph. One pole of the body shows accumulation of dense material (*dm*) in which numerous vesicular profiles are included. Some of these vesicles contain ferritin molecules. The vesicular profiles tend to be grouped in rows which are oriented along the dense material (*dm*). Two of these vesicular profiles (*arrows*) containing ferritin molecules seem to be fused with the membrane of the multivesicular body This organelle shows numerous vesicles which are mainly concentrated in the upper pole. The tracer is found abundantly in the lumen of the multivesicular body. Notice that the tracer is accumulated in small clusters around the inner vesicles but no ferritin molecules are found within the vesicles. Numerous round vesicles (*rv*) without ferritin molecules, are seen adjacent to the membrane of the multivesicular body The tracer appears in dense vesicles (*dv*) small vesicles ( ) and in tubular structure (*t*) located near the multivesicular body 5 3 6


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recorded (Fig. 2). The nature of this potential is still a subject of controversy (Békésy 1952 Butler 1965 Lawrence, 1967) Second when the micropipette impaled the cochlear duct the potential changed polarity (EP) that is the endolymph became positive relative to the reference point in the neck muscle (Békésy 1952) This potential probably is generated by metabolic activity in the stria vascularis (Davis et al. 1958 Tasaki & Spyropoulos, 1959) As shown in Fig 2, the amplitude of EP decayed slowly and progressively in the course of about 5 min from an original value of 76 mV to 70 mV Control experiments without injection of ferritin showed similar decay suggesting that this may be due to factors other than injection of the tracer In this series of experiments no attempt was made to find out whether the magnitude of EP continues to decay in time with or without injection of ferritin

The uptake of ferritin by the endolymphatic cells begins within a few minutes after introducing the marker into the cochlear duct. In all the specimens examined, ferritin molecules were found in the organelles of the endolymphatic cells but not free in their cytoplasm or in the intercellular spaces, basal lamina, perilymphatic cells or perilymphatic spaces (Figs. 5 7) They were found adhering to localized areas of filamentous coating of the outer surface of the cell membrane (Figs. 5 6 7 8, 10 13) or to the coating of different types of vesicular invaginations of the free surface (Figs. 5 6 7 13) and in small invaginations and small uncoated vesicular profiles of the microvilli (Fig. 8).

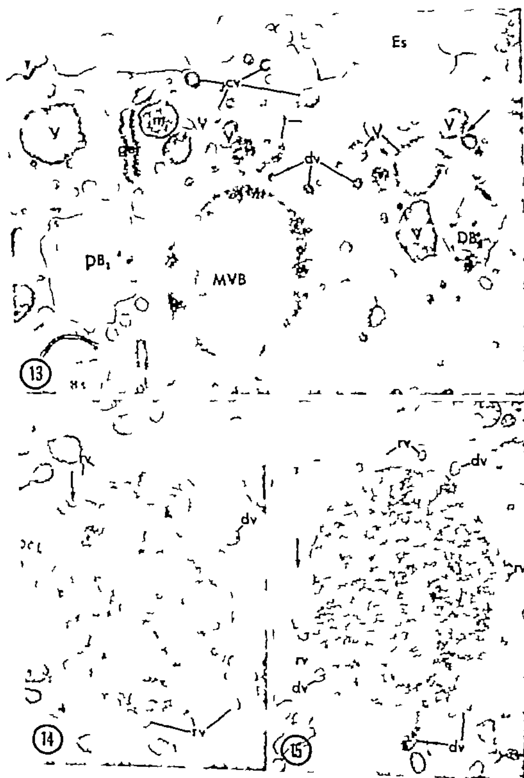
In the cytoplasm, ferritin was observed in different types of vesicles, tubular structures, vacuoles and multivesicular bodies. Vesicles of three types transport the ferritin inside the cytoplasm namely (*a*) coated vesicles which measured between 1 200 Å to 1 800 Å in diameter and in which the molecules were attached to the inner coating of the vesicles (Figs. 5 6 8, 9 13) (*b*) dense vesicles containing somewhat fewer ferritin molecules were characterized by a dense lumen and a diameter between 850 Å to 950 Å and usually located near multivesicular bodies (Figs. 11 12 13 14 15) and (*c*) small uncoated vesicles, measuring between 650 Å to 750 Å in diameter which showed a less dense lumen and usually contained about one to three ferritin molecules. This type of vesicle was located close to vacuoles and multivesicular bodies (Figs. 6 8 10 11 12). The tubular structures are long rods, whose diameter is usually about 950 Å. They have a dense lumen in which some of the tracer's molecules were found. These structures were sometimes continuous with coated vesicles (Figs. 9 10) or

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Fig 13 A portion of the apical cytoplasm of an endolymphatic cell 1 hour after injection of ferritin in the endolymph. A few ferritin molecules (*arrow head*) are seen apparently attached to an area of external coating of the cell membrane. Near the luminal surface there are coated vesicles (*cv*), dense vesicles (*dv*), numerous vacuoles (*V*) and tubular structures (*t*), all containing ferritin molecules. The arrow points toward a coated vesicle which seems to be fusing with a vacuole (*V*). Notice that a large multivesicular body with abundant ferritin molecules, is surrounded by round vesicles. In t pper right side an accumulation of dense material is seen. Dense bodies (*DB* DB_2) show da k round particles and remnants of a membranous st ucture (DB_1). 79 41

Fig 14 Multivesicular body of an endolymphatic cell 2 hours afte njection of ferritin in the endolymph, showing a esicle apparently fusing with or budding from the body (*arrow*). The vesicle is surrounded by dense material. The multivesicular body contains abundant ferritin molecules and a single inner vesicle at the upper pole. No tracer is seen in the round vesicles (*rr*) surrounding the organelle while a dense vesicle (*dv*), located near its membrane contains ferritin molecules. 53 35

Fig 15 Multivesicular body of an endolymphatic cell hours after injection of ferritin in the endolymph. A comparison of the density of concentration of the tracer in this organelle w th those of *Fig. 1* (30 min) and Fig. 13 (60 min) uggest a tendency toward increase of ferritin content and decrease of the number of esicles with increase of time. There is a vesicle apparently fusi g w th or budding from the multivesicular body (*arrow*) Dense material is concentrated at one side of the body (bove the arrow). The multivesicular body is urrounded by numerous round vesicles (*rv*) w thout ferritin ud by dense vesicles (*d*) with ferritin molecules. 47 51


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apparently fused with multivesicular bodies (Fig. 11). More frequently however they were isolated in the cytoplasm (Figs. 8 9 10 12, 13) Vacuoles of various sizes exhibited an inner coating resembling that seen in coated vesicles. Most of the ferritin molecules were embedded in the coating of the vacuole but a few could be found in the lumen (Figs. 9 10 13) The accumulation of ferritin into these vacuoles started approximately 10–20 min after injection of the tracer in the endolymph. Ferritin loaded coated vesicles (Fig. 13 arrow) were seen fused with vacuoles which apparently increased in size and contents (Fig. 13). Some of the small uncoated vesicles (Fig. 8 arrow) were either fused with or budded from, the vacuole. At 30 min multivesicular bodies were seen containing abundant ferritin molecules and numerous inner vesicles (Figs. 5 10 12) In some instances, the inner surface of the membrane of multivesicular bodies showed areas of coating material similar to that found in coated vesicles and vacuoles. The amount of ferritin molecules inside the multivesicular bodies increased progressively with time. At the one hour interval concentration of ferritin increased the multivesicular body was tranformed into a fairly round structure and the inner vesicles were accumulated in a single line along the membrane of the body (Fig. 13) After 2 hours, the concentration of ferritin was still greater the inner vesicles practically disappeared (Fig. 15) An interesting finding in multivesicular bodies was the accumulation of dense material at one pole of the body (Figs. 5 11 12, 13 14 15) This material was projected for a variable distance into the cytoplasm and frequently toward the free surface of the cell. Round and elongated vesicles dense vesicles and tubular structures always containing ferritin molecules, were embedded in this dense material at the polar area of the multivesicular body (Figs. 11 12) where numerous vesicles seemed to be fusing with, or budding from, the membrane of the body (Figs. 11 12, 14 15). Passage of ferritin molecules from one structure to another apparently occurs at this point. Outside this area and surrounding the multivesicular body were numerous small round vesicles 400 to 500 Å in diameter These vesicles contained no ferritin molecules (rv Figs. 5 11 12, 13 14 15) Similar vesicles surrounding multivesicular bodies have been described in macrophages (Cohn et al. 1966 Hirsch et al. 1968) and have been related to the Golgi complex (Novikoff et al. 1964 Brandes et al. 1964 Hirsch et al. 1968)

Perilymphatic injection

Injection of ferritin in the perilymphatic space was also associated with a slow progressive decay of EP^{+} (Fig. 4) The tracer was found in both the perilymphatic and endolymphatic cells as well as in the intercellular spaces, the basal lamina and the perilymphatic and endolymphatic spaces.

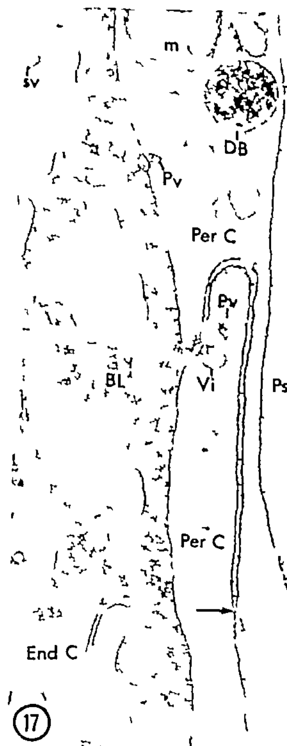
Perilymphatic cell

In all specimens studied the ferritin molecules were seen close to the cell membrane but not attached (Figs. 16 17 18 19 22). The free

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Fig. 16 Reissner's membrane hours after injection of ferritin into the perilymph showing the perilymphatic cell, the basal lamina and the endolymphatic cell The tracer is seen in small clusters directly apposed to the free surface of the perilymphatic cell. This cell shows numerous vesicles (*Pv Pv*) from 400 Å to 1 000 Å in diameter containing ferritin molecules. The basal plasmalemma of the perilymphatic cell shows a vesicular invagination at PVI. Numerous ferritin molecules are found in the basal lamina and intercellular spaces (*arrow heads*) but no ferritin molecules are seen in the zonula occludens of the junctional complex. The endolymphatic cell shows numerous coated vesicles (*cv*), dense vesicles (*dv*), vacuoles (*V*) small vesicles (*rv*), tubular structures (*t*), and a multivesicular body all containing ferritin. The free surface of the endolymphatic cell show ferritin molecules which are attached to small areas of external coating (arrow) and in the coating of vesicular invaginations (*VI*). (*P*) Two cellular compartments of the endolymphatic cell cut transversally 36 936.


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16



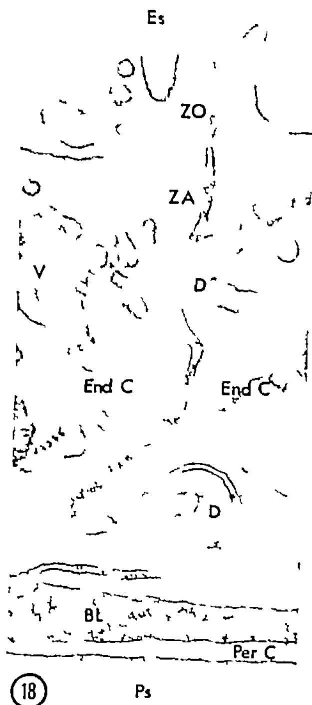


Fig 17 A portion of Reissner's membran hours after injection of ferritin in the pe l mph how ng a junction between two peril mphatic ll Ferritin molecules appear on the luminal surface of the peri lymphatic cells, in the intercellular spaces between perilymphatic cells, in the basal lamina and between endolymphatic cells A *simplified junctional complex* between perilymphat cells is seen at the arrow Ferritin molecules are visible in a vesicular invagi nation (*Vi*), a few esicles (*Pv*) nd a dense body of the perilymphatic cells. The basal portion of an endolymphatic cell shows a small vesicle (*v*) which contains ferritin 55 917

F g 18 A section of Re sner's membrane hours after injection of ferritin into the perilymph show ng a junction between two endolymphatic ll Fer ritin molecules are seen in the per lymphat space near the free surface of the perilymphatic cell, in the basal lamina and i the i tercellular pace be- *tween the endolymphatic cell The trac r appears in* the zonula adherens but the zonula occluden and desmosomes are free of ferritin. An endolymphatic cell shows a vacuole (*V*) with ferr tin 61 560

urface of the perilymphatic cell, unlike that of the endolymphatic cell, exhibited no external coating. The tracer was found also in vesicular invaginations of the apical, lateral and basal plasmalemma of the perilymphatic cell (Figs. 16, 17, 19–22). In the cytoplasm, molecules of the tracer were confined to vesicles, vacuoles and dense bodies. The vesicles, varying from 500 to 1 000 Å in diameter, were located close to the apical and basal cell membranes (Figs. 16, 17, 19) but observed also in the rest of the cytoplasm (Fig. 22). The ferritin was accumulated in vacuoles (Fig. 22) and dense bodies (Fig. 17) but the concentration in the latter appeared to be the same after one or two hours since injection.

Basal lamina and intercellular spaces

In the basal lamina the ferritin molecules were accumulated in great quantity which varied directly with the time since injection. Ferritin was found in the intercellular spaces between perilymphatic cells (Fig. 17) and between endolymphatic cells (Figs. 16, 17, 18, 19–22) including the zonula adherens of their junctional complex. The tracer did not cross the zonula occludens (Figs. 16, 18).

Endolymphatic cell

The basal plasmalemma and the lower third of the lateral cell membrane of the endolymphatic cell exhibited a few small vesicular invaginations containing numerous ferritin molecules (Figs. 19–22, arrows). No coated invaginations were seen in either the basal plasmalemma or lateral cell membrane. In the cytoplasm, molecules of the tracer were found in small vesicles, dense vesicle, coated vesicles, tubular structures, vacuoles and multivesicular bodies (Figs. 16, 18, 19–22). Comparison of perilymphatic and endolymphatic injection experiments, showed a differential distribution of ferritin. In the former, the organelles containing ferritin were distributed throughout the cytoplasm while in the latter the organelles containing ferritin were distributed only in the upper third of the cell. Tracer was also discovered in vesicular invaginations of the free surface of the endolymphatic cell (Figs. 16, 19) as well as in small vesicular invaginations of the microvilli (Figs. 20, 21). Furthermore, ferritin was seen either free in the endolymphatic space (Fig. 21) or apparently attached to the external filamentous coating of the free surface of the cell membrane (Fig. 16).

Discussion

The observations presented in this report indicate that macromolecules of ferritin were transported across Reissner's membrane from perilymph to endolymph. There was no transport of ferritin across Reissner's membrane from endolymph to perilymph, although the macromolecules were taken up by the endolymphatic cell.

Perilymphatic injection

Fig. 23 serves as a model for our assumptions regarding transport of ferritin across Reissner's membrane from perilymphatic to endolymphatic spaces. The tracer reaches the basal lamina through the intercellular space between the perilymphatic cells. The few small uncoated invaginations found in the basal, apical and lateral plasmalemma of the perilymphatic cell and the small uncoated vesicles within the cell, all containing ferritin, suggests that the tracer may also reach the basal lamina by means of a transcellular transport. A feature observed in the perilymphatic cell is the absence of areas of filamentous coating on the free surface, and the absence of

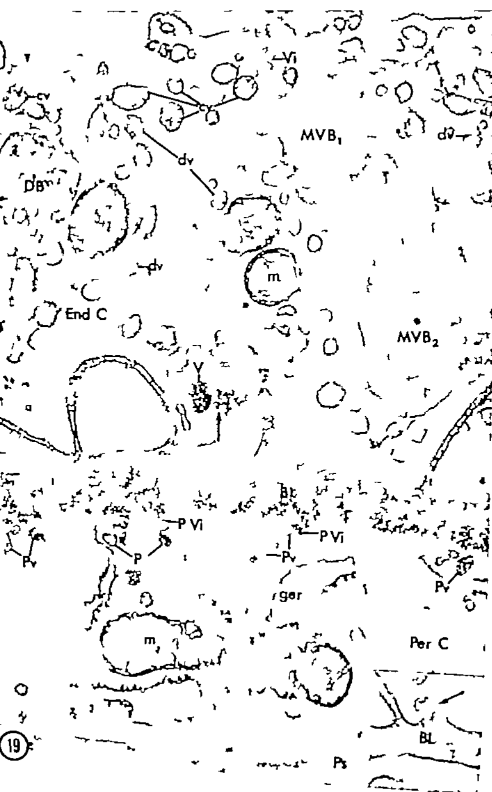
coated invaginations and coated vesicles. Perhaps the latter are related to the absence of areas of filamentous coating this may however be a fixation artifact.

Although the ferritin may be transported across the perilymphatic cell, the evidence, clearly indicates that the ferritin reaches the basal lamina predominantly by diffusion through intercellular spaces. The molecules are accumulated in the basal lamina and intercellular spaces between the endolymphatic cells up to but not within the zonula occludens. Since the zonula occludens seems to be a barrier for diffusion of ferritin, how can one explain its presence in the endolymph? Perhaps it could have resulted from a rupture of the Reissner's membrane during injection of ferritin into the perilymph. This, however is unlikely because the pressure applied to the injected fluid was negligible. Reissner's membrane can be distorted considerably before it ruptures. A more convincing argument against rupture of Reissner's membrane rests on the fact that the endocochlear potential was maintained at near normal level during and, for at least 5 min after injection of ferritin in either perilymph or endolymph A rupture of Reissner's membrane results in a mixture of perilymph and endolymph followed by a significant fall in the amplitude of the endocochlear potential (Davis et al. 1955) The observed slight decline of EP may be attributed to local damage produced by displacement of the micropipette relative to the organ of Corti that could have occurred during respiration of the animal The physiological evidence and the characteristic pattern distribution of ferritin after injection in the perilymph compared with the pattern exhibit after injection in the endolymph, to be discussed below strongly suggests that there was no contamination of endolymph with perilymph. Rather the tracer was transported across the endolymphatic cell. A question of considerable theoretical importance is how the tracer was absorbed by the endolymphatic cell transported across it and then discharged into the endolymph Although it is not valid to draw conclusions regarding dynamic relationships from electron micrographs, there are however some observations which support the processes outlined in the model (Fig. 23)

We assume that the absorption of ferritin was done by small uncoated invaginations which are consistently found along the basal plasmalemma and lower third of the lateral cell membrane. Although the tracer was diffused through the intercellular spaces up to the zonula occludens, no invaginations appeared in the upper two-thirds of the lateral plasmalemma. There was no morphological differences in the basal and lateral plasmalemma of the endolymphatic cell which may account for the specializations of invaginations mentioned above The observation that coated invaginations were never found in the basal and lateral plasmalemma suggests that the uptake of the tracer may be accomplished by small uncoated vesicles.

Regarding the transport of ferritin across the endolymphatic cell we assume a migration

Fig 19 Reissner's membrane tw hours afte njection of ferritin in the p.rilymph *P l mphatic c ll* The tracer appears in the p rilymphatic space near the free surface of the cell (th luminal plasmalemma was cut tangentially) N merous vesicles (*Pv*) from 500 A to 1 000 A in diameter containing ferritin, are seen close to th basal plasmalemma. Two vesicular in aginations of the basal plasmalemma are seen at PVi. Basal lamina and i tercellular spaces contain numerous ferritin molecules. *Endolymphatic cell.* Ferritin molecules are found in a vesicular invagination (*arro*) of the lower portion of the lateral plasmalemma. A vacuole (*I*) nd a f w vesicles located near this vesicular invaginat on also contain ferr tin The tracer is also found in one small vesicle (*n*), dense esicle (*d*), coated esicle ( ), tubular structures (*t*) and mult esicular bodies (*M B* *M B*). Notice the amorphous dense content of the mult vesicular body MVB. The f ee surface of the cell show ferritin molecules t a small tangentially ut area (*arrow head*) and in a vesicular in gination (*I I*) *Inset* The basal plasmalemma of the endolymphatic cell show a esicular in gination with ferrit n molecules (*arrow*). *54 891* *Inset* *65 151*


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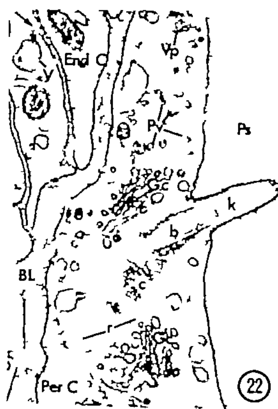
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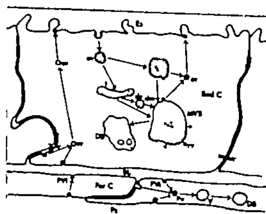
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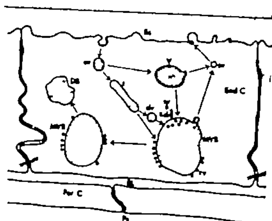
Regarding the transport of ferritin across the endolymphatic cell we assume a migration

Fig. 19 Reissner's membrane two hours after injection of ferritin in the perilymph. *Perilymphatic cell.* The tracer appears in the perilymphatic space near the free surface of the cell (the luminal plasmalemma was cut tangentially). Numerous vesicles (*PV*) from 500 Å to 1 000 Å in diameter containing ferritin are seen close to the basal plasmalemma. Two vesicular invaginations of the basal plasmalemma are seen at *PVi*. *Basal lamina and intercellular spaces* contain numerous ferritin molecules. *Endolymphatic cell* Ferritin molecules are found in a vesicular invagination (*arrow*) of the lower portion of the lateral plasmalemma. A vacuole (*V*) and a few vesicles located near this vesicular invagination also contain ferritin. The tracer is also found in one small vesicle (*sv*), dense vesicle (*dv*), coated vesicles (*cv*), tubular structures (*t*) and multivesicular bodies (*MVB*, *MVB*). Notice the amorphous dense content of the multivesicular body MVB. The free surface of the cell shows ferritin molecules at a small tangentially cut area (*arrow head*) and in a vesicular invagination (*VI*). *Inset* The basal plasmalemma of the endolymphatic cell shows vesicular invagination with ferritin molecules (*arrow*). 54 891 *Inset* 65 151

sv
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20
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of small uncoated vesicles from base to the free surface. This assumption is reasonable because no ferritin was ever found free in the cytoplasm while small uncoated vesicles were seen throughout the cell. The distribution of these vesicles contrasts with the distribution of coated and small uncoated vesicles after injection of ferritin in the endolymph. In this case the small vesicles and other organelles containing ferritin are always located in the upper third of the cell. The transcellular transport of ferritin across the endolymphatic cell from perilymph to endolymph appears similar to that described by Bruns & Palade (1968) in the endothelial cells of the muscle capillaries. In their study the ferritin was injected in the saphenous vein The transcellular transport of the tracer by the endothelial cell was done by "plasmalemma vesicles which to a certain extent appear similar to our small uncoated vesicles. Other transcellular transport has been described in the endothelial cells of the glomerulus of the kidney by Farquhar & Palade (1962) in a number of endothelial cells, Kupffer cells and phagocytic cells in the spleen by Florey (1967) and in mesothelial cells by Odor (1956)

We assume that ferritin discharge into the endolymph is accomplished by fusion of small uncoated vesicles with the luminal plasmalemma as a reverse pinocytosis (de Duve & Wattiaux, 1966) Figures 16 and 21 show two types of invagination one coated the other uncoated. The features of these two invaginations regarding size location and coating suggest that they represent different processes. The uncoated invaginations are small, and unlike the coated ones, are consistently found at the base of the microvilli. The functional significance of their location is not apparent. On the other hand the experimental evidence from various sources (Roth & Porter 1962, Roth & Porter 1964 Fawcett 1964 Rosenbluth & Wissig 1964) indicates that the coated invaginations represent a specific process for uptake of proteins. In agreement with this concept the ferritin transported from perilymph to endolymph is again absorbed by a mechanism similar to that following injection of ferritin into the endolymph That is, the discharged ferritin is reabsorbed by coated invaginations which are found along the luminal plasmalemma. Then the small uncoated invaginations resulting, we presume, from fusion of small uncoated vesicles with the luminal plasmalemma remains as one alterna

Fig ?0 A portion of the apical cytoplasm of an endolymphatic cell two hours after injection of ferritin into the perilymph showing the tracer in numerous small vesicles (*sv*) and a dense vesicle (*dv*). The arrow points toward a small vesicle which seems to be open into the endolymphatic space however this may be debatable because the area was cut tangentially Numerous ferritin molecules are seen in the endolymphatic space near the cell membrane. 77 976

Fig 21 An area of the apical cytoplasm of an endolymphatic cell two hours after i jection of ferritin into the perilymph. The arrow points tow rd a small vesicular invagination at the base of a microvillus. Ferritin molecules a seen in the vesicular invagination, along the cell membrane and n the endolymphatic space The t ace s also found in a small esicle (*v*), a dense esicle (*d*) and in n apparent vesicular structure (*x*), with ill-defined li miting membrane. 6 586

Fig 2 Portion of Reissner's membrane hours after injection of ferriti in the perilymphatic space showing the tracer near the free surface in numerous vesicles (*Pv*), a acuole (*Vp*), basal lamina and intercellular spaces. The basal portion of the lateral plasmalemma of the endolymphatic cell shows a vesicular invagination containing ferrit n (*arro*). The tracer is also found in a vacuole (*V*). An interesting feature shown in this figure but seen only in a f w perilymphatic cells was the existence of a structure with the characteristics of a kinocilium (*k*) with a basal body (*b*), a centriole (*c*), and rootlets (*r*). The functional significance of this structure is not appar ent. Ferritin molecules are seen on the free surface of the kinocilium but not in its matrix. ?9 754

F g 3 A diagrammatic summary of Reissner membrane fter injection of ferritin in the perilymphatic space The model i based on the interpretation of the electron micrographs obtained in this study and the reader should refer to these figures nd th text for details The abbreviations represent the same structures as in the figures.

Fig 4 This model presents in diagrammatical form the events in Reissner membrane after in tion of ferritin in the endolymphatic pace Refer to the text for the discussion of these events.

ments. *The question is now how the small uncoated vesicles are formed after injection of ferritin in the endolymph.* One possibility is that they originate from small vesiculations in areas of the multivesicular body's membrane which are uncoated and perhaps from the vacuoles. The association of these organelles with small uncoated vesicles can be seen in Figs. 8, 12, 14 and 15 which also show uncoated vesicles containing ferritin in the space between luminal plasmalemma and both vacuoles and multivesicular bodies.

Recapitulating, the uptake of ferritin by the perilymphatic cell was clearly demonstrated in the micrographs but transport across the cell was not evident. On the other hand the endolymphatic cell seems to be provided with two surfaces possessing different properties regarding uptake of ferritin. The basal and lateral lower third plasmalemmas act as a specialized area for absorption of macromolecules. The membrane of these areas are deprived of coated material and the process of absorption seems to take place by means of uncoated invaginations which in turn formed uncoated vesicles transporting ferritin across the cell. The tracer was discharged into the endolymph by fusion of uncoated vesicles with luminal plasmalemma at the base of the microvilli. *The other surface of the endolymphatic cell, the luminal plasmalemma,* is able to absorb macromolecules by coated vesicles and also discharge the molecules back into the endolymph by a process of reversed pinocytosis. The molecules absorbed by the luminal plasmalemma are not transported across the cell but they were accumulated in organelles located in its upper third. Some molecules were discharged back into the endolymph by fusion of uncoated vesicles with the plasmalemma located at the base of the microvilli. The process *of discharge of the tracer into the endolymph seems to be the same whether* the molecules were absorbed by the basal or by luminal plasmalemmas. *A final* commentary regarding the results of this investigation, is that it provides a preparation which can be used for study transport of a variety of macromolecules across an epithelial membrane under physiological control.

Acknowledgment

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Summary

Transport of ferritin across Reissner's membrane was studied with electron microscopy in the cat. Measurement of endocochlear potential served as control of the functional state of the cochlea. Injection of ferritin in endolymph showed uptake of ferritin molecules by coated invaginations and accumulation within numerous organelles of the endolymphatic cell. No tracer was found in the extracellular spaces, basal lamina or perilympatic cells.

After injection *of ferritin in the perilymph,* the tracer was found *in both perilymphatic* and endolymphatic cells, *intercellular* spaces and endolymph. *Ferritin* was absorbed by the basal and lateral plasmalemmas of the endo-

tive for explaining the discharge of ferritin into the endolymph.

Endolymphatic Injection

After injection of ferritin into the endolymph the uptake by the endolymphatic cell seems to follow in general, the classical stages of pinocytosis described by several authors (Bennett 1956 Bessis & Breton-Gorius, 1957 Brandt & Pappas, 1960 Bennet 1963 Choi 1963 Bowers, 1964 Fawcett, 1964 Fawcett, 1965 Holter 1965 Hirsch et al. 1968) The model representing the uptake and subsequent stages is outlined in Fig. 24 The molecules are absorbed by localized areas of filamentous coating found along the luminal plasmalemma. These areas exhibit structural features comparable to those already described in the literature (Brandt & Pappas, 1960 Bennett, *1963 Fawcett, 1964 1965 Holter 1965)* Invaginations of these specialized areas form the classical coated invaginations. Another coated structure associated with the luminal surface seen occasionally with the electron microscope was the channel-like invaginations. These long wide structures are coated along their length. There was no formation of smaller vesicles which could be associated with these channel-like invaginations. It should be noted that ferritin was always found in these structures but the fate of both ferritin and channel like invaginations was not clear in the specimens. Channel like invaginations have been described in the amoebae (Chapman Andresen & Prescott 1956 Holter 1965) and in the lung phagocytes after injection of India ink (Karrer 1960) As is well known the coated invaginations gives place to forma tion and separation of coated vesicles. The next step seems to be the formation of vacuoles by fusion of coated vesicles. The tubular structures appear also to originate from or are associated with coated vesicles as shown in Figs. 9 and 10 The question whether the tubular structures may be also formed directly from invaginations of the luminal plasmalemma remains open The electron micrographs failed to show a contribution of tubular structures to the formation of vacuoles as shown by Bowers (1964) in the pericardial cells *of an insect. The enlargement* of vacuoles by further fusion with coated vesicles forms the multivesicular bodies and perhaps tubular structures may contribute to their formation by fusion or indirectly via dense vesicles. The diameter and density of the lumen of both tubular structures and dense vesicles suggests that the latter could represent tubular structures sectioned transversally as shown in Fig 11 Apparently the multivesicular body follows several stages regarding loss of inner coating and concentration of both ferritin molecules and inner vesicles. A point of interest in our material is the presence of dense material which was repeatedly found associated with the area of fusion of tubular structures and dense vesicles with the multivesicular body As suggested by others (Novikoff & Shin 1964 Ericsson et al. 1965 Wood, 1965) the final stage of the multivesicular body could be the dense body An interesting observation in our material was the presence of small uncoated invaginations found at the base of the microvilli (see the inset of Fig. 8) These invaginations contain ferritin molecules and the question is whether the invagination represents a process for uptake or for discharge of ferritin Because the formation is uncoated and routinely found in relation with the microvilli as in the case of ferritin injected into the perilymph we tentatively interpret it as a process *for discharge of the tracer into the* endolymph. If this interpretation is correct how are the small uncoated invaginations formed when ferritin is injected into the endolymph? The presence of small uncoated vesicles containing ferritin in the neighborhood of uncoated invaginations suggests that the latter may represent a fusion of uncoated vesicles with luminal plasmalemma The process for discharging ferritin into the endolymph could be identical in the two sets of experi

ments. The question is now how the small uncoated vesicles are formed after injection of ferritin in the endolymph. One possibility is that they originate from small vesiculations in areas of the multivesicular body's membrane which are uncoated and perhaps from the vacuoles. The association of these organelles with small uncoated vesicles can be seen in Figs. 8, 12, 14 and 15 which also show uncoated vesicles containing ferritin in the space between luminal plasmalemma and both vacuoles and multivesicular bodies.

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After injection of ferritin in the perilymph, the tracer was *found* in both perilymphatic and endolymphatic cells, intercellular spaces and endolymph. Ferritin was absorbed by the basal and *lateral* plasmalemmas of the endo-

lymphatic cell and it was accumulated in various cell organelles. It was also found in coated and uncoated invaginations of the endolymphatic cell free surface. These findings indicate that transport of ferritin takes place across Reissner's membrane from perilymph toward endolymph but not in the opposite direction.

Zusammenfassung

Die Beförderung von Ferritin durch Reissnersche Membran wurde mittels Elektronenmikroskopie an Katzen untersucht. Messung des endokochlearen Potentials diente als Kontrolle für den Funktionszustand der Kochlea. Ferritininjektionen in die Endolymphe zeigte Aufnahme von Ferritinmolekülen von überzogenen Invaginationen und Ansammlung in zahlreichen Organellen der endolymphatischen Zelle. Es wurde kein Spurenmaterial in extrazellulären Zwischenräumen der Basalschicht oder perilymphatischen Zellen festgestellt.

Nach Ferritininjektionen in die Perilymphe, wurde Spurenmaterial sowohl in perilymphatischen als auch/in endolymphatischen Zellen festgestellt in Zwischenzellenraumen und in der Endolymphe. Ferritin wurde von basalen und seitlichen Plasmalemmae der endolymphatischen Zelle aufgenommen und wurde in verschiedenen Zellorganen angesammelt. Es konnte ebenfalls in überzogenen und unüberzogenen Invaginationen der freien Oberfläche der endolymphatischen Zelle festgestellt werden. Diese Resultate weisen darauf hin, dass die Beförderung von Ferritin durch Reissnersche Membran sich von der Perilymphe zur Endolymphe vollzieht nicht aber aus entgegengesetzter Richtung.

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lymphatic cell and it was accumulated in various cell organelles. It was also found in coated and uncoated *invaginations of the endolymphatic cell free surface.* These findings indicate that transport of ferritin takes place across Reissner's membrane from perilymph toward endolymph but not in the opposite direction.

Zusammenfassung

Die Beförderung von Ferritin durch Reissnersche Membran wurde mittels Elektronenmikroskopie an Katzen untersucht. Messung des endokochlearen Potentials diente als Kontrolle für den Funktionszustand der Kochlea. Ferritininjektionen in die Endolymphe zeigte Aufnahme von Ferritinmolekülen von überzogenen Invaginationen und Ansammlung in zahlreichen Organellen der endolymphatischen Zelle. Es wurde kein Spurenmaterial in extrazellulären Zwischenräumen der Basalschicht oder perilymphatischen Zellen festgestellt.

Nach Ferritininjektionen in die Perilymphe, wurde Spurenmaterial sowohl in perilymphatischen als auch/in endolymphatischen Zellen festgestellt in Zwischenzellenraumen und in der Endolymphe. Ferritin wurde von basalen und seitlichen Plasmalemmae der endolymphatischen Zelle aufgenommen und wurde in verschiedenen Zellorganen angesammelt. Es konnte ebenfalls in überzogenen und unüberzogenen Invaginationen der freien Oberfläche der endolymphatischen Zelle festgestellt werden. Diese Resultate weisen darauf hin, dass die Beförderung von Ferritin durch Reissnersche Membran sich von der Perilymphe zur Endolymphe vollzieht, nicht aber aus entgegengesetzter Richtung.

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CONTENTS

Introduction

The termination within the vestibular nuclei of the first order neurons supplying the vestibular sense organs has recently been demonstrated (Gacek, 1969). The axons of the second order neurons comprising these vestibular nuclei project to [1] the nuclei of the extraocular muscle nerves, [2] the anterior horn cells of spinal cord levels, and [3] the cerebellum. These form the basis for the well-known vestibular reflexes. The first of these three reflex connections (eye responses) has attracted the most interest, both on a clinical and a laboratory level, probably because it is the most visible and accessible to precise measurement.

Accurate knowledge of the anatomical connections serving the vestibulo-ocular reflex is important for proper understanding of the physiology of this reflex in normal and abnormal states. The lack of such information was recently pointed out by Brodal (1962), (pp. 127). "Although some data are available, too little is as yet known of the detailed anatomy of the connections of the vestibular nuclei with the nuclei of the extrinsic ocular muscles to permit closer anatomico-physiological correlations." The purpose of the present study is to describe the course and precise termination of second order neurons in the vestibular nuclei which project to the III IV and VI cranial nerve nuclei. These connections were demonstrated by tracing axonal degeneration of these neurons produced by lesions in the vestibular nuclei of the cat. Descending projections were also studied but will be discussed in a general way in this report.

n the *IV and III nucleus* of the same side. This tract was thought to originate in the abducens nucleus and not the vestibular nuclei.

In 1904 van Gehuchten described the main two ascending projections from the vestibular complex. On the basis of extensive lesions in and around Deiters nucleus in the rabbit, he concluded that:

1 ascending fibers in the contralateral MLF come by way of the inferior arcuate fibers either from "the terminal nucleus" of the vestibular nerve or from the acoustic tubercle (dorsal cochlear nucleus).

2 ascending fibers in the ipsilateral MLF come by way of the superior arcuate fibers from the nucleus of Bechterew (superior vestibular nucleus).

Muskens in 1914 produced a very complete analysis of the degeneration patterns resulting from lesions throughout the vestibular system. He described three ascending tracts to the ocular nuclei:

1 A contralateral group of fibers that occupied the dorsomedial aspect of the MLF and supplied the IV and III nuclei. The origin of this system was the nucleus triangularis—the term he applied to the ventral caudal division of Deiters nucleus.

2 An ipsilateral system that traveled in the lateral part of the MLF and terminated primarily in the IV and III nuclei with a more rostral termination probable but not determinable in these studies. The superior vestibular nucleus was the sole source of this group of fibers.

3 An ipsilateral system which traveled lateral to the most lateral part of the MLF and which could be traced to midbrain levels but not beyond. He felt that the medium-sized cells in Deiters dorsal magnocellular division gave rise to this tract.

Lloyd Gray in 1926 presented a clear analysis of his results following electrolytic lesions in the vestibular nuclei of the cat. As concerns the ascending connections he described:

1 A contralateral MLF projection to the IV and III nuclei and the interstitial nucleus of Cajal and Darkschewitsch nucleus as well. He felt certain that the origin of this system was the rostral medial vestibular nucleus. This tract also terminated on the opposite abducens (VI) nucleus by way of collaterals of the axons from the medial vestibular nucleus passing into the contralateral MLF.

2 An ipsilateral MLF projection to IV III nuclei, interstitial nucleus of Cajal and Darkschewitsch nucleus. The superior vestibular nucleus was the sole source of this group of fibers.

A. T. Rasmussen (1932) in the cat described:

1 A contralateral ascending system in the MLF which projected to the levels of the IV and III nuclei. He felt the origin of this group to be either the medial or the descending vestibular nuclei since his lesions were not small enough to permit a more precise statement as to the source.

2 An ipsilateral ascending tract which occupied the lateral half of the MLF and distributed to the IV and III nuclei of the same side. He confirmed the origin of this system from the superior vestibular nucleus.

3 On the basis of his lesions he could not find any ascending projections from the lateral (Deiters) vestibular nucleus.

In 1937 Buchanan in an extensive study of lesions in the vestibular nuclei of the cat critically described the ascending and descending projections. He demonstrated three ascending projections as did Muskens:

1 A contralateral system in the MLF which arose mainly from the medial vestibular nucleus (nucleus triangularis in his terminology). A small number of fibers also was described in the ipsilateral MLF from the same source. The ultimate termination of this system to the midbrain eye nuclei were vaguely referred to but definite statement was made that the contralateral VI (abducens) nucleus received collaterals from this contralateral system. The lateral vestibular (Deiters) nucleus was also felt to contribute to this contralateral system.

Review of Literature

Many electrophysiological studies have already been reported showing the response of different eye muscles to peripheral sense organ stimulation. The reports of Bender (1964) Cohen and co-workers (1964) and Fluur (1959) are cited as examples of this type of investigation Essentially information from such studies have shown that characteristic eye movements in different planes correspond to sense organs that are stimulated by a force in the same plane Such results represent the entire three neuron arc from periphery to the effector muscle—in this case the extra-ocular muscles (Szentagothai 1950)

Precise information on connections can only be *obtained* by studying one neuron at a time. A *physiological* attempt at this is exemplified by the recent study of Tokumasu Goto & Cohen (1969) where a stimulating electrode was placed at various points of the vestibular *complex of* the monkey and produced eye movements which were somewhat different in the various regions of the nuclear complex. In their study a stimulating electrode placed in the superior vestibular nucleus produced upward rotatory eye movements, nystagmus with upward rotatory slow phases and with downward rotatory quick phases some horizontal eye movements but no downward eye movements. Stimulation of the mid portion of the vestibular complex produced upward rotatory horizontal and downward rotatory eye movements. Placement of the stimulating electrode at the junction of the rostral poles of the medial and descending vestibular nuclei produced mostly downward rotatory eye movements and nystagmus with downward rotatory slow phases and upward rotatory quick phases. An indication of the need for precise anatomical data of the second order neurons was given by their statement that "nothing is known about projections from this area" when referring to the junction of the rostral poles of the medial and descending vestibular nuclei.

Anatomical demonstration of these projections can be accomplished by the method of (*a*) axonal degeneration following lesions at the cells *of origin* or (*b*) retrograde cell changes produced by transection of the main axons of these cells. Of the two methods, the axonal degeneration technique is preferable because

(*a*) axons will regularly degenerate following injury to its cell body or axon while with the retrograde method the reaction in the cell body following a peripheral injury to its axon is not always predictable and is more difficult to assess accurately

(*b*) precise termination on cell groups within the extraocular nuclei can only be studied by staining the fiber terminals.

The most recognized comprehensive early anatomical studies of the vestibulo-ocular projections were those of Fraser (1901) Gehuchten (1904) Muskens (1914) Gray (1926) Rasmussen (1932) and Buchanan (1937)

On the basis of degeneration patterns following fairly extensive lesions in the medial longitudinal bundle and Deiter s nucleus in monkeys and cats, Fraser (1901) described two ascending projections to the midbrain

1 A contralateral system which coursed in the dorso-medial aspect of the MLF and terminated in the IV and III nuclei and the nucleus of Darkschewitsch of that side The origin of this group of fibers, he concluded, was Deiters nucleus.

2. An ipsilateral system which traveled in the lateral wing of the MLF and terminated

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after large lesions in the superior vestibular nucleus of the cat, that the degeneration oc curred in the medial cell group of the contralateral oculomotor (III) complex which in turn innervated the ipsilateral superior rectus muscle. He also described terminal degeneration in the ventral cell column of the ipsilateral oculomotor complex which innervated the ipsilateral medial rectus muscle. He concluded that these projections to the ipsilateral superior rectus and medial rectus from the superior vestibular nucleus corresponded to the termination of the superior and horizontal semicircular canals respectively within that nucleus, thus providing evidence of an organization to the projection of these sense organs through the superior nucleus to the eye nuclei. Also described were terminal degeneration in the trochlear and oculomotor nuclei following very large lesions in the lateral (Deiters) vestibular nucleus corresponding to otolith-ocular reflex pathways.

The second study was that of Carpenter (1966) in which he reported the degeneration patterns in the MLF and the III, IV and VI extraocular nuclei following large electrolytic lesions limited to each of the vestibular nuclei in the monkey. Essentially he found that: (1) the superior vestibular nucleus projected in an entirely ipsilateral fashion in the MLF and terminated in the ipsilateral III IV and VI nuclei. In the oculomotor complex he found all subdivisions including that supplying the inferior rectus muscle received fibers from the superior vestibular nucleus. (2) the medial vestibular nucleus projected bilaterally to the VI nucleus, and while it projected in the ascending MLF mainly in a contralateral fashion there was also a lesser ipsilateral ascending projection as well. Therefore, termination in the IVth and IIIrd nuclei was bilateral but more pronounced to the contralateral oculomotor (III) nucleus, specifically the intermediate and ventral-lateral cell groups which innervate the inferior oblique and medial rectus. In the ipsilateral oculomotor (III) complex the termination of the ipsilateral projection involved the dorsal and ventral-lateral cell columns which innervate the inferior rectus and the medial rectus respectively. These ascending systems were noted to terminate further in both of the interstitial nuclei of Cajal. (3) The lateral vestibular nucleus projected bilaterally to the abducens (VI) nuclei and to the contralateral ascending MLF similar to the projection of the medial nucleus. Ipsilateral termination in the dorsal-lateral cell column (inferior rectus) and the ventral-lateral cell group (medial rectus) of the oculomotor (III) complex was also described.

While both these studies carefully studied the terminal portions of the ascending systems, they both utilized large lesions produced electrically to the estibular nuclei and therefore could not correlate termination with origin accurately. The lack of clear uniformity and agreement on the origin and termination of the vestibulo-ocular projections remained.

The third study of vestibulo-ocular projections published very recently is that of Tarlov (1970). He has clearly shown an ipsilateral projection to the extraocular nuclei from the superior vestibular nucleus and a contralateral one from the rostral part of the medial vestibular nucleus. No mention of the ascending tract of Deiters was made in this study. He also inflicted lesions in the vestibular nuclei with the electrolytic method but carefully evaluated the size and location of these lesions and could accurately determine the origin of the two MLF systems. He was *not* able to demonstrate a correlation *of different regions of the* superior or medial vestibular *nuclei* to individual extraocular muscle.

In the *present study small* discrete lesions in the vestibular *nuclei of the* cat were produced with a one millimeter diameter probe *without the* use of electric current. After a short survival time of 6 to 7 days to allow for Wallerian degeneration, the animals were sacrificed and the Nauta silver method used to study the degeneration patterns in the brainstem and midbrain. *Origin* and termination *of* three vestibulo-ocular systems were clarified in this manner

2. An ipsilateral system in the MLF to midbrain levels which projected from the superior vestibular nucleus.

3 An ipsilateral tract lateral to the MLF which projected to midbrain levels and arose from the rostral two-thirds of Deiters (lateral vestibular) nucleus. Again termination was not precisely described to the midbrain eye nuclei but the VI (abducens) nuclei of both sides received fibers from Deiters nucleus.

A definite statement was also made by Buchanan that no ascending projections could be attributed to the descending vestibular nucleus.

In summarizing these classical anatomical studies one might say that the vestibular complex of one side is connected to the extra ocular nuclei (IV and III in particular) of both sides of the midbrain by way of both medial longitudinal fasciculi and possibly a third tract (ascending tract of Deiters) which is not contained in the MLF The important data concerning these tracts that have not been determined are (*a*) their precise origin in the vestibular nuclei (with the exception of the superior vestibular nucleus which is the undisputed sole source of the ipsilateral ascending MLF system) (*b*) their precise ultimate termination within the extra-ocular nuclei and other midbrain centers.

There are several reasons why this information is lacking despite the carefully executed investigations of these anatomists. Two factors contributed to the disagreement as to the source of the contralateral tract in the MLF and the so-called "ascending tract of Deiters

(*a*) The lesions produced in this complex area where several (*medial, lateral and descending*) of the vestibular nuclei adjoin or overlap had been extensive and usually involved more than one nucleus to some extent Limitation of the size of the lesion is very difficult when the electrolytic method is used The spread of current beyond the area of visible tissue necrosis may cause neuronal degeneration that is misleading in interpretation. In all these studies except for that of Rasmussen electric current was used to produce lesions. Although Rasmussen used a specially constructed small knife the lesions produced were extensive

(*b*) nomenclature and definition of the vestibular nuclei in this caudal area of the vestibular complex has differed. This usually has involved the terms nucleus triangularis and Deiters nucleus Muskens and Fraser referred to the ventral Deiters (lateral) which contained a layer of small and medium cells as the "nucleus triangularis while to other anatomists (Rasmussen Buchanan) nucleus triangularis clearly referred to the medial vestibular nucleus. Deiters nucleus refers in a strict sense to the lateral vestibular nucleus as identified by large or medium large multipolar cell bodies. Yet to those who use the term Deiters nucleus" in a broad sense it means an area defined by arbitrary boundaries which may include the smaller cells of an adjoining or overlapping nucleus such as the medial vestibular nucleus (see Figs. 4 5)

Information on the termination of these fiber systems is deficient for a very obvious reason All of these experiments reviewed utilized the Marchi method for selectively demonstrating axonal degeneration. This method of course relied on the osmification of free fat particles in degenerating myelin. Therefore only substantially myelinated degenerating fibers are reliably demonstrated The thinly myelinated or unmyelinated terminal portions of long axons escaped observation.

The Nauta silver method which was introduced in 1954 (Nauta, 1954 1957) selectively demonstrated even the finest terminal parts of both myelinated and unmyelinated neurons. The obvious tremendous advantage over the Marchi method led to a new era in neuroanatomical investigation

Three studies on the termination in the extraocular nuclei of ascending vestibular neurons utilizing the Nauta method have appeared

The report of Szentagothai (1964) indicated

after large lesions in the *superior vestibular* nucleus of the cat, that the degeneration occurred in the medial cell group of the contralateral oculomotor (III) complex which in turn innervated the ipsilateral superior rectus muscle. He also described terminal degeneration in the ventral cell column of the ipsilateral oculomotor complex which innervated the ipsilateral medial rectus muscle. He concluded that these projections to the ipsilateral superior rectus and medial rectus from the *superior* vestibular nucleus corresponded to the termination of the superior and horizontal semicircular canals respectively within that nucleus, thus providing evidence of an organization to the projection of these sense organs through the superior nucleus to the eye nuclei. Also described were terminal degeneration in the trochlear and oculomotor nuclei following very large lesions in the lateral (Deiters) vestibular nucleus corresponding to otolith-ocular reflex pathways.

The second study was that of Carpenter (1966) in which he reported the degeneration patterns in the MLF and the III, IV and VI extraocular nuclei following large electrolytic lesions limited to each of the vestibular nuclei in the monkey. Essentially he found that (1) the superior vestibular nucleus projected in an entirely ipsilateral fashion in the MLF and terminated in the ipsilateral III, IV and VI nuclei. In the oculomotor complex he found all subdivisions including that supplying the inferior rectus muscle received fibers from the superior vestibular nucleus. (2) the medial vestibular nucleus projected bilaterally to the VI nucleus, and while it projected in the ascending MLF mainly in a contralateral fashion there was also a lesser ipsilateral ascending projection as well. Therefore, termination in the IVth and IIIrd nuclei was bilateral but more pronounced to the contralateral oculomotor (III) nucleus, specifically the intermediate and ventral-lateral cell groups which innervate the inferior oblique and medial rectus. In the ipsilateral oculomotor (III) complex the termination of the ipsilateral projection involved the dorsal and ventral-lateral cell columns which innervate the inferior rectus and the medial rectus respectively. These ascending systems were noted to terminate further in both of the interstitial nuclei of Cajal. (3) The lateral vestibular nucleus projected bilaterally to the abducens (VI) nuclei and to the contralateral ascending MLF similar to the projection of the medial nucleus. Ipsilateral termination in the dorsal-lateral cell column (inferior rectus) and the ventral-lateral cell group (medial rectus) of the oculomotor (III) complex was also described.

While both these studies carefully studied the terminal portions of the ascending systems, they both utilized large lesions produced electrically to the vestibular nuclei and therefore could not correlate termination with origin accurately. The lack of clear uniformity and agreement on the origin and termination of the vestibulo-ocular projections remained.

The third study of vestibulo-ocular projections published very recently is that of Tarlov (1970). He has clearly shown an ipsilateral projection to the extraocular nuclei from the superior vestibular nucleus and a contralateral one from the rostral part of the medial vestibular nucleus. No mention of the ascending tract of Deiters was made in this study. He also inflicted lesions in the vestibular nuclei with the electrolytic method but carefully evaluated the size and location of these lesions and could accurately determine the origin of the two MLF systems. He was not able to demonstrate a correlation of different regions of the superior or medial vestibular nuclei to individual extraocular muscle.

In the present study small discrete lesions in the vestibular nuclei of the cat were produced with a one millimeter diameter probe without the use of electric current. After a short survival time of 6 to 7 days to allow for Wallerian degeneration, the animals were sacrificed and the Nauta silver method used to study the degeneration patterns in the brainstem and midbrain. Origin and termination of three vestibulo-ocular systems were clarified in this manner.

2. An ipsilateral system in the MLF to midbrain levels which projected from the superior vestibular nucleus.

3 An ipsilateral tract lateral to the MLF which projected to midbrain levels and arose from the rostral two-thirds of Deiters (lateral vestibular) nucleus. Again termination was not precisely described to the midbrain eye nuclei but the VI (abducens) nuclei of both sides received fibers from Deiters nucleus.

A definite statement was also made by Buchanan that no ascending projections could be attributed to the descending vestibular nucleus.

In summarizing these classical anatomical studies one might say that the vestibular complex of one side is connected to the extraocular nuclei (IV and III in particular) of both sides of the midbrain by way of both medial longitudinal fasciculi and possibly a third tract (ascending tract of Deiters) which *is not contained in the MLF* The important data concerning these tracts that have not been determined are (*a*) their precise origin in the vestibular nuclei (with the exception of the superior vestibular nucleus which is the undisputed sole source of the ipsilateral ascending MLF system) (*b*) their precise ultimate termination within the extra-ocular nuclei and other midbrain centers.

There are several reasons why this information is lacking despite the carefully executed investigations of these anatomists. Two factors contributed to the disagreement as to the source of the contralateral tract in the MLF and the so-called ascending tract of Deiters

(*a*) The lesions produced in this complex area where several (medial lateral and descending) of the vestibular nuclei adjoin or overlap had been extensive and usually involved more than one nucleus to some extent Limitation of the size of the lesion is very difficult when the electrolytic method is used. The spread of current beyond the area of visible tissue necrosis may cause neuronal degeneration that is misleading in interpretation. In all these studies except for that of Rasmussen electric current was used to produce lesions. Although Rasmussen used a specially constructed small knife the lesions produced were extensive.

(*b*) nomenclature and definition of the vestibular nuclei in this caudal area of the vestibular complex has differed. This usually has involved the terms nucleus triangularis and Deiters nucleus Muskens and Fraser referred to the ventral Deiters (lateral) which contained a layer of small and medium cells as the nucleus triangularis while to other anatomists (Rasmussen Buchanan) "nucleus triangularis clearly referred to the medial vestibular nucleus. "Deiters nucleus refers in a strict sense to the lateral vestibular nucleus as identified by large or medium large multipolar cell bodies. Yet to those who use the term Deiters nucleus in a broad sense it means an area defined by arbitrary boundaries which may include the smaller cells of an adjoining or overlapping nucleus such as the medial vestibular nucleus (see Figs. 4 5)

Information on the termination of these fiber systems is deficient for a very obvious reason All of these experiments reviewed utilized the Marchi method for selectively demonstrating axonal degeneration This method of course relied on the osmification of free fat particles in *degenerating myelin*. Therefore only substantially myelinated degenerating fibers are reliably demonstrated The thinly myelinated or unmyelinated terminal portions of long axons escaped observation

The Nauta silver method which was introduced in 1954 (Nauta 1954 1957) selectively demonstrated even the finest terminal parts of both myelinated and unmyelinated neurons. The obvious tremendous advantage over the Marchi method led to a new era in neuroanatomical investigation

Three studies on the termination in the extraocular nuclei of ascending vestibular neurons utilizing the Nauta method have appeared

The report of Szentagothai (1964) indicated

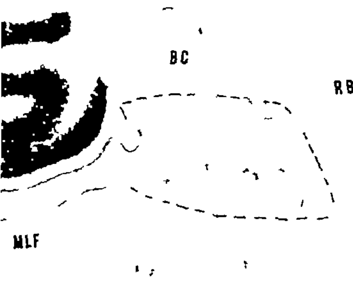


Fig. 2. Level A. Fig. 1. Transverse section through the superior vestibular nucleus (outlined area). Note proximity to brachium conjunctivum (*BC*) and restiform body (*RB*).

indicated in level C (Fig. 4) and ( ) the fact that incoming first order neurons terminate only in the ventral portion of the lateral vestibular nucleus.

Brodal (1960) in particular has demonstrated the fact that the projection to the spinal cord from this nucleus is a very highly organized one with the most rostral and ventral levels projecting to cervical levels and the dorsal and more caudal levels to the lowest portion of the cord with the intervening areas distributing in a corresponding way to other levels of the spinal cord.

Descending vestibular nucleus (DVN)

The descending or inferior vestibular nucleus is the more lateral of the two caudal vestibular nuclei, the more medial one appropriately named the medial vestibular nucleus (MVN). The descending vestibular nucleus (DVN) is most easily recognized at very caudal levels such as the photomicrograph taken at level C (Fig. 6), by the fact that its cells are interspersed among the fibers of the descending vestibular root. However the most rostral limit of the descending vestibular nucleus is somewhat more difficult to precisely delimit. The descending nucleus is immediately caudal to the ventral division of the lateral vestibular nucleus and, indeed, does undercut it in a fashion similar to the way that the lateral vestibular nucleus adjoins the superior vestibular nucleus described in the preceding section. The medial limit, however of the DVN at the most rostral pole is left mainly to individual interpretation. If one uses the dorsal acoustic stria (DAS) as the lateral boundary of the MVN at rostral levels then the smaller celled area which is just lateral to the DAS and adjacent to the ventral lateral vestibular nucleus may be included either in the DVN or in the MVN (Fig. 5.) Indeed in previous work (Gacek, 1969) on the incoming first order neurons and their sites of termination this area was referred to as belonging to the DVN. As will be pointed out later in this study it perhaps is more justifiable to include this area as part of the medial vestibular nucleus since its projection is similar to that of the medial vestibular nucleus and not at all similar to the

Preliminary to describing the experimental portion of this study a short discussion on the important anatomical considerations of the vestibular nuclei as well as of the III, IV and VI nuclei will be presented

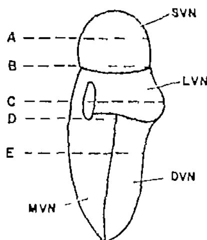


Fig 1 Outline drawing of the four major vestibular nuclei, plan view Levels *A* through *E* indicate location of Figs. through 6 which represent transverse sections through the nuclei. The clear oval area left of center marks position of the dorsal acoustic stria. *SVN* Superior vestibular nucleus *LVN* Lateral vestibular nucleus *DVN* Descending vestibular nucleus, *MVN* Medial vestibular nucleus.

VESTIBULAR NUCLEI

An outline drawing of the vestibular complex is shown in Fig. 1 Levels A through E indicate levels through the vestibular complex which will be described with accompanying photomicrographs of normal cat material stained with the thionine method for demonstrating neurons.

Superior vestibular nucleus (SVN)

The superior vestibular nucleus is the most rostral of the four main vestibular nuclei and is comprised of larger cells in the center of the nucleus and smaller cells at the periphery Fig. 2 is taken through level A and shows the cell arrangement characteristic of this nucleus. The incoming ascending branches of the first order neurons from the cristae of the semicircular canals course obliquely through the nucleus from ventrolateral to dorsomedial Axons of the cells comprising the nucleus emerge from the nucleus at the medial aspect of its rostral pole to course in a medial direction Almost all previous work on fiber pathways indicate that the cells of this nucleus project in an entirely ipsilateral fashion in the MLF to the oculomotor and trochlear nuclei

Lateral vestibular nucleus (LVN)

The lateral vestibular nucleus is immediately caudal to the superior vestibular nucleus and in fact its most rostral limit undercuts the caudal end of the superior vestibular nucleus in the manner indicated in the photomicrograph taken through level B (Fig 3) As indicated in photomicrographs taken through levels C and D (Figs. 4 5) the lateral vestibular nucleus is characterized by the giant multipolar neurons comprising this nucleus although medium and small cells populate some of the more ventral and caudal areas of the nucleus. The nucleus is bounded medially by the dorsal acoustic stria (DAS) and laterally by the restiform body and the incoming vestibular root fibers. Many investigators have felt that this nucleus can be divided into two divisions, a ventral and a dorsal one for two reasons. (1) the obvious anatomical division

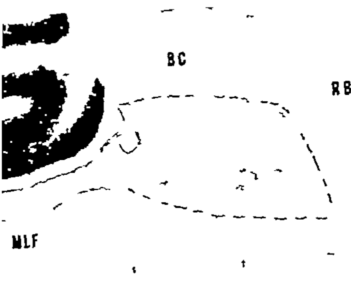


Fig. 2. Level A Fig. 1. Transverse section through the superior vestibular nucleus (outlined area). Note proximity to brachium conjunctivum (*BC*) and restiform body (*RB*).

indicated in level C (Fig. 4), and (2) the fact that incoming first order neurons terminate only in the ventral portion of the lateral vestibular nucleus.

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Anatomy of Vestibular and Ocular Nuclei

Preliminary to describing the experimental portion of this study a short discussion on the important anatomical considerations of the vestibular nuclei as well as of the III IV and VI nuclei will be presented

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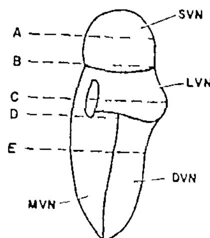


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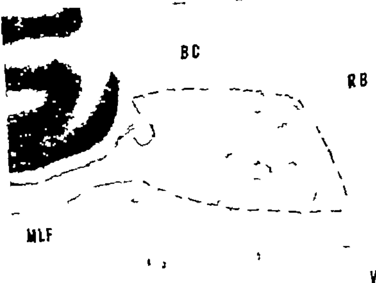


Fig. 2. Level *A* Fig. 1. Transverse section through the *superior vestibular* nucleus (outlined area). Note proximity to brachium conjunctivum (*BC*) and restiform body (*RB*).

indicated in level C (Fig. 4), and (2) the fact that incoming first order neurons terminate only in the ventral portion of the lateral vestibular nucleus.

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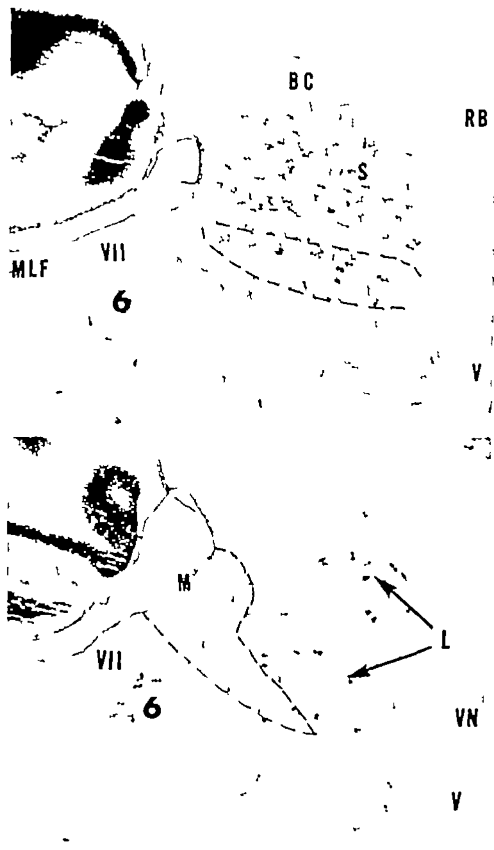


Fig 3 Level *B* Fig. 1 Transverse section through the caudal end of the superior vestibular nucleus (*S*) showing the most rostral limit of the lateral vestibular nucleus (outlined area) *VII* = facial nerve *V* = descending trigeminal root

Fig 4 Level *C* Fig. 1 Transverse section through the midportion of the lateral vestibular nucleus (*L*) showing the dorsal and central division (ar) of this nucleus and relationship to the medial vestibular nucleus (*M*) in the outlined area. At this level the abducens nucleus (6) and nerve are also seen ventral to the facial genu (*VII*) VN vestibular nerve root



Fig. 5. Level *D*, Fig. 1. Transverse section through the caudal portion of the lateral vestibular nucleus (*L*). The arrow indicates the area of smaller cells which is included in the medial vestibular nucleus (M) rather than the lateral or descending vestibular nuclei. *DAS* – dorsal coustic stria.

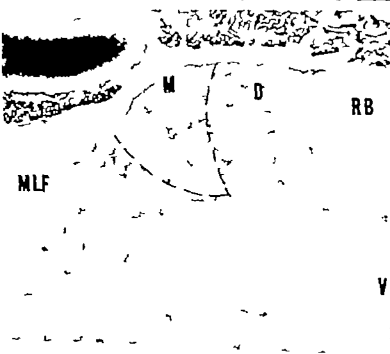


Fig. 6. Level *E*, Fig. 1. Transverse section through the medial (*M*) and descending (*D*) vestibular nuclei. The larger cells of the descending nuclei are separated by the fiber bundles of the descending vestibular root.

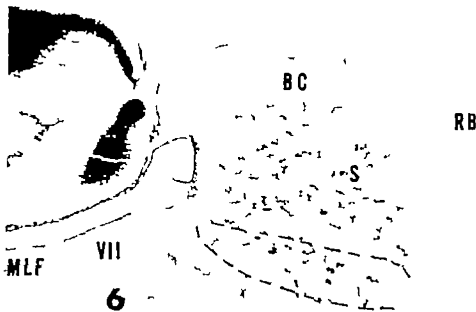


Fig 3 Level *B* F'g 1 Transverse section through the caudal end of the su perior vestibular nucleus (*S*) showing the most rostral limit of the lateral vestibular nucleus (outlined area). *VII* = facial nerve *V* = descending trigeminal root.

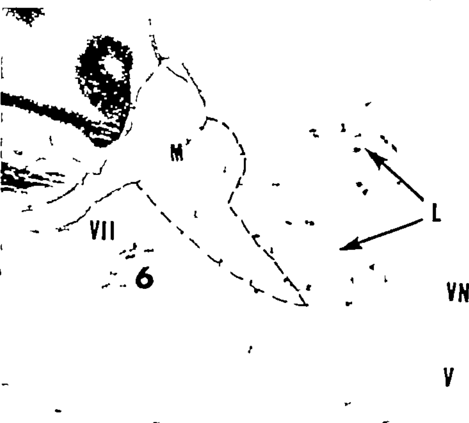


Fig 4 Level *C* Fig. 1 Transverse section through the midportion of the lat eral vestibular nucleus (*L*) showing the dorsal and entral divisions (*arro s*) of this nucleus and relationship to the medial estibular nu leus (*M*) in the outlined a ea. At this level the abducer nu leus (*6*) nd nerve are also seen entral to the facial genu (*VII*) V V est bul r nerve root

ABDUCENS (VI) NUCLEUS

The abducens or sixth cranial nerve nucleus is comprised of a group of rather large motor neurons which occupy a region immediately below the genu of the facial nerve (Fig. 4). The nucleus extends in a longitudinal direction so that it reaches from the rostral limit of the lateral vestibular nucleus to the rostral portions of the descending vestibular nucleus. The axons of these neurons project in a directly ventral direction and they innervate the ipsilateral lateral rectus muscle. It is important to remember that the dendrites of these motor neurons, as has been shown by Cajal (1909–11) and Lorente de Nó (1926), extend for considerable distances and indeed even reach into the substance of the medial vestibular nucleus. Therefore, degeneration that is described in the vicinity of the motor neuron itself is usually a conservative estimate because degeneration may be present on the dendrites of these neurons and yet be indistinguishable from degeneration within the MVN itself. The abducens is the only ocular nerve nucleus which is located in relation to the floor of the fourth ventricle.

TROCHLEAR (IV) NUCLEUS

This nucleus is comprised of fairly large motor neurons which make up a spherical nucleus that indents the dorsal surface of the MLF in a very characteristic manner (Fig. 7). This nucleus is located in the midbrain and innervates the contralateral superior oblique muscle after emerging from the dorsal surface of the midbrain. It is the smallest of the extraocular nerve nuclei both in area and in number of cells.



Fig. 7. Transverse section through the trochlear (*4*) nuclei showing the characteristic indenting of the medial longitudinal fasciculus (*MLF*).

projections of the descending nucleus. This, therefore will be included as a portion of the medial vestibular nucleus and not the descending

The outflow of the descending vestibular nucleus has been described in previous studies as projecting to cerebellar regions and in a descending direction to spinal cord by way of both the contralateral and ipsilateral MLF Several authors have indicated there is no evidence of ascending projections to the eye nuclei from the descending vestibular nucleus. (Buchanan 1937 Carpenter 1966 1960)

Medial vestibular nucleus (*MVN*)

The medial vestibular nucleus again is depicted in photomicrographs through level C through E (Figs. 4–6) It makes up the most medial half of the caudal end of the vestibular complex and is the longest nucleus of the group It parallels the length of both the lateral vestibular nucleus and the descending vestibular nucleus. Its makeup is mainly of small and medium-sized cell bodies and output is regarded as both in an ascending and a descending direction by way of the contralateral MLF



Fig. 9. Transverse section through the caudal third of the oculomotor nucleus. The medial cell columns (larger outlined area) is most prominent of the cell groups at this level, although the dorso-lateral group (smaller outlined area) can be seen also. Cf. Fig. 8. *RN* = red nucleus.

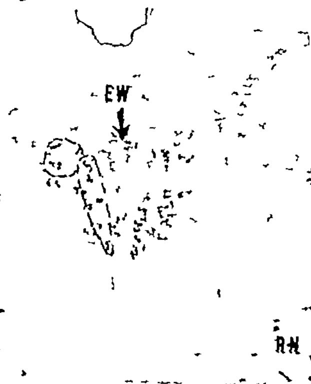


Fig. 10. Transverse section through the middle third of oculomotor complex. The medial column is smaller and the lateral cell groups are more prominent. Note the differentiation of 3 cell groups on the right-hand side of the nucleus. Cf. Fig. 8. *EW* = Edinger Westphal nucleus.

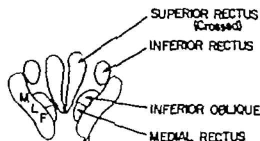


Fig. 8. Diagram of the subdivisions of the oculomotor nucleus complex. All groups except the medial column (superior rectus) innervate the ipsilateral eye muscles indicated.

OCULOMOTOR (III) NUCLEUS

The oculomotor complex is the largest and most complicated of the eye nuclei. It is located in the midbrain below the sylvian aqueduct and is immediately rostral to the IV or trochlear nucleus. The nuclear subdivisions which innervate the four extraocular muscles innervated by the III nerve have been described in the monkey by Warwick (1953) and this classification will be utilized in this study (Fig. 8). At most caudal levels through the oculomotor complex (Fig. 9) one can see primarily the medial cell column which has been shown by Warwick to innervate the contralateral superior rectus muscle. At this point the lateral cell column is not as prominent but can be seen much more clearly in the middle and rostral areas of the nuclear complex. Through the midportion of the oculomotor complex (Fig. 10) the medial cell column is less extensive while the lateral cell column is more prominent and can be seen to present into at least two subgroups—a dorsal and a ventral one. The dorsal group has been shown to innervate the inferior rectus muscle. The ventral group has been further subdivided in the monkey (Warwick) (this is not as clear in the cat) into a more dorsal group which is called the intermediate group and innervates the inferior oblique and a most ventral group which innervates the medial rectus muscle. All the cell groups in the lateral cell column innervate the ipsilateral muscle groups indicated. At the most rostral level (Fig. 11) through the oculomotor complex the medial cell column and the dorsal group of the lateral cell column are not seen and a small ventral group of the lateral cell column can be seen. This group innervates the medial rectus muscle primarily. The two other nuclei important to this study since they represent the most rostral terminus of the vestibulo-ocular projection are the nuclei associated with the MLF rostral to the oculomotor complex. These are the nucleus of Darkschewitsch (ND) and the interstitial nucleus of Cajal (IC). These are relatively condensed accumulations of small cells. The nucleus of Darkschewitsch is located dorso-lateral to the MLF and the interstitial nucleus of Cajal is more ventro-laterally located in relation to this medial longitudinal bundle. These are illustrated in Fig. 12.

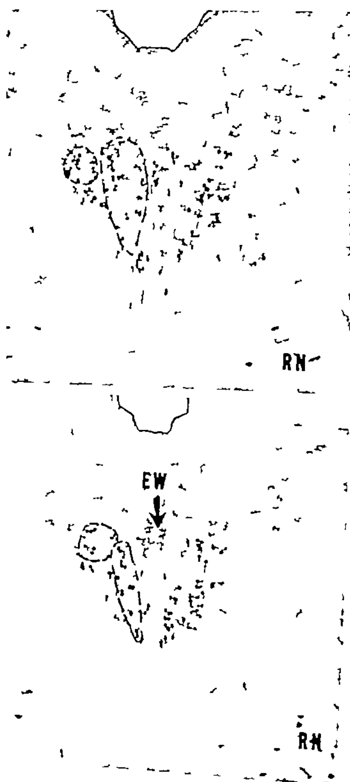


Fig. 9 Transverse section through the caudal third of the oculomotor nucleus. The medial cell columns (larger outlined area) is most prominent of the cell groups at this level, although the dorso-lateral group (smaller outlined area) can be seen also. Cf. Fig. 8. *RN* = red nucleus.

Fig. 10 Transverse section through the middle third of oculomotor complex. The medial column is smaller and the lateral cell groups are more prominent. Note the differentiation of 3 cell groups on the right-hand side of the nucleus. Cf. Fig. 8. *EW* = Edinger-Westphal nucleus.

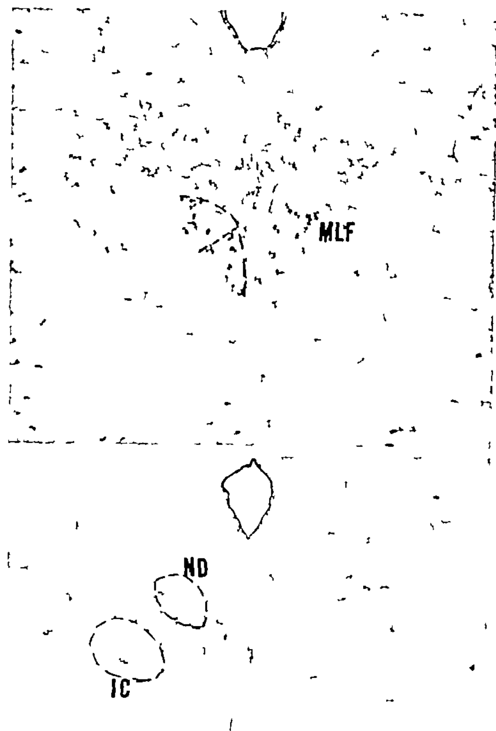


Fig. 11. Transverse section through the rostral third of the oculomotor nucleus. At this level the medial cell column and the dorsolateral group are not visible and only the intermediate-ventral lateral group (outlined) is recognizable.

Fig. 12. Transverse section rostral to the oculomotor nucleus showing the nucleus of Darkschewitsch (*ND*) and the interstitial nucleus of Cajal (*IC*).

Materials and Methods

Thirty-five healthy young adult cats were used in this study. After adequate intraperitoneal injection of Nembutal (30 mg per kg), the skull and back of the neck were shaved and prepped for surgical procedure. A midline skin incision separating the posterior neck muscles and removal of the occipital portion of the bone from the foramen magnum to the lambdoidal crest was then carried out with rongeurs in order to obtain exposure of the posterior fossa. After careful incision and reflection of dura and arachnoid, the right side of the cerebellar hemisphere and vermis were carefully elevated from the dorsal surface of the brainstem with the use of the operating microscope. Care was used to avoid interruption of the vascular supply. This elevation was carried out with a malleable metal strip retractor until the dorsal acoustic stria coursing medially from the dorsal cochlear nucleus could be seen as the principal landmark. The most medial extent of the dorsal acoustic stria served as a landmark for the vestibular nuclei. At this point if an animal was to serve as a control no further surgery was performed. If the animal was to be used as an experimental lesion animal, then a straight probe of 1 mm diameter was inserted in a rostro-ventral direction to a predetermined depth in order to produce a lesion in the intended vestibular nucleus on the right side. For lesions in the rostral part of the vestibular complex, that is the superior vestibular nucleus, the probe was inserted dorsal and rostral to the dorsal acoustic stria and passed in a rostro-ventral and slightly medial direction. If lesions were to be made in the more caudal aspects of the vestibular complex lesions, the probe entered the dorsal surface of the vestibular area, caudal and ventral to the dorsal acoustic stria in a similar direction. The probe was withdrawn and the operative defect closed by placing a piece of gelfoam over the exposed area of cerebellum and approximating the occipital and temporal muscles loosely to allow for postoperative expansion of the cerebellum. The skin closure was performed in the usual manner with interrupted sutures.

Animals were not administered antibiotics but were given 5% glucose and water the first postoperative day.

Detailed recordings of eye movements in these animals were not obtained. With the exception of one or two animals with more extensive lesions, no spontaneous gross eye movements were noted. Therefore, electronystagmographic recording of ocular movement would be necessary. Such instrumentation was not available.

In addition, short survival tissues of 6–7 days were necessary to optimally demonstrate degenerating nerve terminals in the ocular nuclei. Cerebellar edema from retraction in the posterior fossa surgical approach introduces another factor in the animal's functional deficit during the immediate postoperative period. Therefore, interpretation of recordings during this period would be most difficult.

After a survival period of 6 or 7 days the animals were perfused intravitally with ten percent formalin with three percent potassium dichromate added. The calvarium was removed and the dura reflected in order to allow further fixation by immersion in 10% formalin. This was accomplished by placing the head and brain in a jar with 500 cc of 10% neutralized formalin. After two to three



Fig. 11. Transverse section through the rostral third of the oculomotor nucleus. At this level the medial cell column and the dorsolateral group are not visible and only the intermediate-ventral lateral group (outlined) is recognizable.

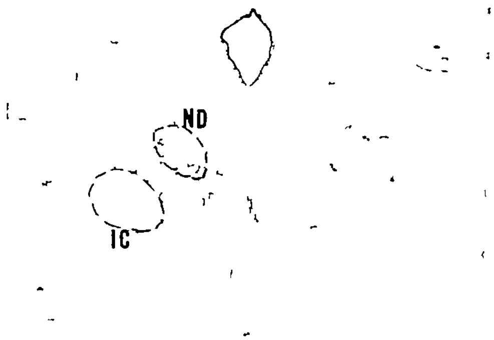


Fig. 1[illegible] Transverse section rostral to the oculomotor nucleus showing the nucleus of Darkschewitsch (*ND*) and the interstitial nucleus of Cajal (*IC*).

Observations

A. CONTROL ANIMALS

(a) The brainstems of three cats, in which operative exposure alone of the vestibular nuclei was carried out, were carefully examined. No degeneration was found in the extraocular nuclei of all three animals. Therefore, the posterior fossa approach for producing vestibular nuclear lesions was satisfactory for this study

(b) Fig. 13 summarizes the lesion and resulting degeneration pattern in animal no. 92. The lesion was produced by inserting the probe through the dorsal division of the lateral (Deiters) vestibular nucleus rostrally along the dorsal border of the superior vestibular nucleus. Here the central portion of the brachium conjunctivum was involved and the superior vestibular nucleus spared (Fig. 14).

There was no degeneration in either MLF The degenerating fibers of the brachium conjunctivum could be followed across the midline at the level of the interpeduncular nucleus and into the contralateral Red Nucleus where heavy terminal degeneration was found. However close examination revealed many fine degenerating fibers in small groups emanating dorsally from the area of the Red Nucleus. These fibers pierced the contralateral MLF at right angles (see Fig. 15) in the area of the III and IV nuclei. Terminal degeneration was clearly found in the medial cell group of the contralateral III nucleus (Fig. 16) and in the rostral and dorsal rim of the contralateral IV nucleus (Fig. 17). This pattern and distribution of degenerating fibers from lesions in brachium conjunctivum was clearly and constantly found in all five animals in this group

This finding confirmed the presence of a direct cerebello-ocular projection in the cat. Knowledge of distribution of this projection permitted accurate evaluation of lesions in the periphery of the superior vestibular nucleus where the brachium conjunctivum was also involved.

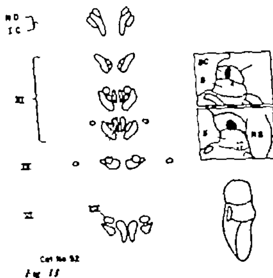


Fig. 13

B. EXPERIMENTAL ANIMALS

Although all twenty-four experimental animals with lesions in the vestibular nuclei were reconstructed, ten such reconstructions will be described in detail since they show the significant ascending projections to the extraocular nuclei.

The lesions and degeneration patterns are graphically illustrated in a summary diagram for each animal described in the text. The lesion is located by a solid black area in its rostro-caudal extent on the drawing in the lower right corner and in its dorsoventral location on outlines of selected sections through

days of fixation by immersion the brainstems were removed under the operating microscope to avoid trauma or traction on the cranial nerves emerging from the brainstem and mid brain areas. This part of the brain was further fixed by immersion in ten percent formalin for 48 hours before portions of the brainstem and midbrain were blocked and readied for sectioning. Frozen sections were cut at 30 μ and were then processed with the Nauta technique of silver staining.

Sections of each animal were examined carefully and reconstructed to depict the lesion and degeneration patterns emanating from the lesion in each animal.

Of the total of thirty-five animals, three animals died in the postoperative period for unknown reasons and twenty-four were utilized as experimental animals with lesions in the vestibular nuclei. The other eight cats served as the control group

Two significant points were studied in the control group

1 It was considered possible that degeneration in the extraocular nuclei could be produced by traction or pressure on the brainstem nuclei or cerebellum during the operative procedure. Three cats were operated upon in the manner previously described to expose the vestibular nuclear area but no intentional lesion made with the probe. After the usual 6–7 day survival period, the brainstems and ocular nuclei were examined in these animals.

2. Direct cerebello-ocular fiber projections through the brachium conjunctivum have been described in several animal species (Allen 1924 Carpenter 1964 Van Gehuchten 1905 Gerebtzoff 1936 1941 Klimoff 1896 1899 Rasmussen 1932)

Since the brachium conjunctivum is immediately adjacent to the superior vestibular nucleus and is frequently involved in lesions of this nucleus, the presence and termination of this projection was studied in five cats. These cats represented those in which the probe had missed its intended target in the superior vestibular nucleus and involved different portions of the brachium conjunctivum superior to the nucleus.

Observations

A. CONTROL ANIMALS

(a) *The brainstems of three cats, in which operative exposure alone of the vestibular nuclei was carried out, were carefully examined.* No degeneration was found in the extraocular nuclei of all three animals. Therefore, the posterior fossa approach for producing vestibular nuclear lesions was satisfactory for this study

(b) Fig. 13 summarizes the lesion and resulting degeneration pattern in animal no. 92. The lesion was produced by inserting the probe through the dorsal division of the lateral (Deiters) vestibular nucleus rostrally along the dorsal border of the superior vestibular nucleus. Here the central portion of the brachium conjunctivum was involved and the superior vestibular nucleus spared (Fig. 14).

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This finding confirmed the presence of a direct cerebello-ocular projection in the cat. Knowledge of distribution of this projection permitted accurate evaluation of lesions in the periphery of the superior vestibular nucleus where the brachium conjunctivum was also involved.

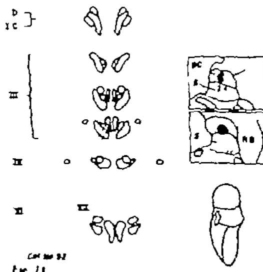


Fig. 13

B EXPERIMENTAL ANIMALS

Although all twenty-four experimental animals with lesions in the vestibular nuclei were reconstructed, ten such reconstructions will be described in detail since they show the significant ascending projections to the extraocular nuclei.

The lesions and degeneration *patterns* are graphically illustrated *in a summary diagram* for each *animal* described in the text. The lesion is located by a solid black area in its rostro-caudal extent on the drawing in the lower right corner and in its dorsoventral location on outlines of selected sections through

Fig. 14. Nauta stained transverse section through the superior vestibular nucleus (*S*) in Cat 9 demonstrating the lesion (*arrow*) in the brachium conjunctivum (*BC*).

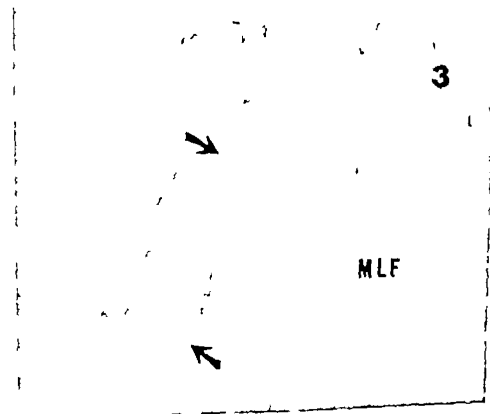


Fig. 15. High power photomicrograph through the contralateral MLF near the oculomotor nucleus (*3*) of Cat 9. Arrows point out fine degenerating nerve fiber coursing from the area of the Red Nucleus, passing through the MLF to terminate in the contralateral oculomotor nucleus. Cf. Fig. 13.

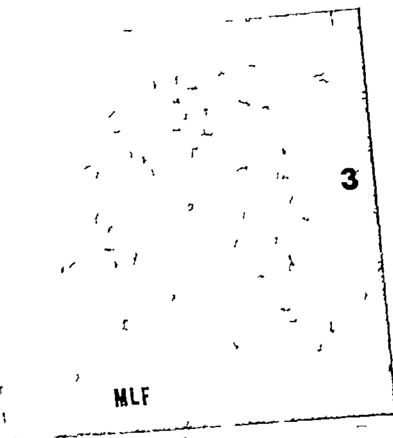


Fig. 16 High power view through the contralateral oculomotor nucleus of same animal demonstrating the terminal degeneration in the medial cell column. Cf. Fig. 13



Fig. 17 Terminal degeneration (*arrow*) in the dorsal aspect of the contralateral trochlear (*4*) nuclear of Cat 9... Cf. also Fig. 13

the vestibular nuclei at the right upper and middle portions of the diagram. Degenerating fibers are represented by dashed lines emanating from the lesion area in the outline of the vestibular complex at lower right. The six drawings in the center column represent the cross-section outlines of the MLF and the extraocular nuclei and midbrain nuclei as identified by the labels at left. Degenerating fibers coursing in the MLF are shown as heavy dots and terminal degeneration within the nuclei as a fine stippled area

I *Lesions in the superior vestibular nucleus* Lesions produced in the superior vestibular nucleus were made by passing the probe in a ventro-medial direction after inserting the probe above the dorsal acoustic stria. This lesion necessarily involved the dorsal division of the lateral vestibular nucleus in all cases (Figs. 18 25 31 36) However as determined in this study and those of others (Buchanan 1937 Carpenter 1966) lesions in this portion of the lateral vestibular nucleus did not contribute to any ascending degeneration in the brainstem Degeneration from lesions in this portion of the lateral nucleus was entirely in a descending direction by way of the ipsilateral vestibulo-spinal tract. Therefore all ascending degeneration produced in this manner could be correlated with the area of superior vestibular nucleus involved.

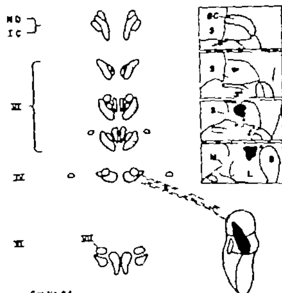


Fig 18.

Cat no. 84 (see Fig 18)

In this animal the lesion involved the most caudal and medial portion of the superior vestibular nucleus (Fig. 19). Since the axons of the cells in the intact rostral portion of the nucleus (Fig. 20) leave the rostral pole of the nucleus, the degeneration pattern in this animal correlated with the caudal-medial part of the SVN

Degenerating nerve fibers of all sizes were followed in a rostro-medial direction from the SVN and entered the lateral wing of the ipsilateral MLF at the rostral level of the fourth ventricle. These degenerating fibers retained a lateral position in the MLF throughout the midbrain. Collaterals were observed as these fibers passed the IV and III nuclei. Terminal degeneration was significant in the ipsilateral IV nucleus (Fig. 21). In the III nuclear complex terminal degeneration was heaviest in the ipsilateral intermediate-lateral group although a lesser amount of degeneration also was found in the ipsilateral ventral cell mass. (Fig. 22 *A B*) There were also degenerating fibers crossing the midline (Fig. 23) within the oculomotor complex to terminate in the inter mediate and ventral lateral cell column of the contralateral side to a lesser degree. The medial cell group and the dorsal-lateral cell group of both sides of the oculomotor com-

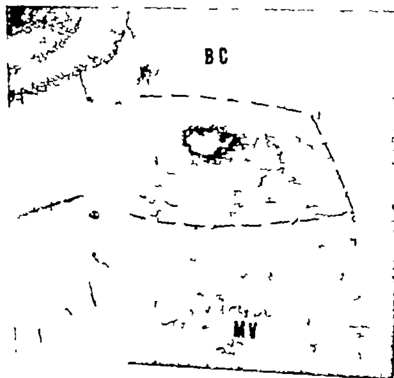


Fig 19 Transverse section through the caudal half of the superior vestibular nucleus showing the lesion in the medial portion of the nucleus. Cat, 84. *MV* = motor nucleus of trigeminal nerve.

the vestibular nuclei at the right upper and middle portions of the diagram. Degenerating fibers are represented by dashed lines emanating from the lesion area in the outline of the vestibular complex at lower right. The six drawings in the center column represent the cross-section outlines of the MLF and the extraocular nuclei and midbrain nuclei as identified by the labels at left. Degenerating fibers coursing in the MLF are shown as heavy dots and terminal degeneration within the nuclei as a fine stippled area.

I. *Lesions in the superior vestibular nucleus*
Lesions produced in the superior vestibular nucleus were made by passing the probe in a ventro-medial direction after inserting the probe above the dorsal acoustic stria. This lesion necessarily involved the dorsal division of the lateral vestibular nucleus in all cases (Figs. 18, 25, 31, 36). However as determined in this study and those of others (Buchanan 1937, Carpenter 1966) lesions in this portion of the lateral vestibular nucleus did not contribute to any ascending degeneration in the brainstem. Degeneration from lesions in this portion of the lateral nucleus was entirely in a descending direction by way of the ipsilateral vestibulo-spinal tract. Therefore all ascending degeneration produced in this manner could be correlated with the area of superior vestibular nucleus involved.

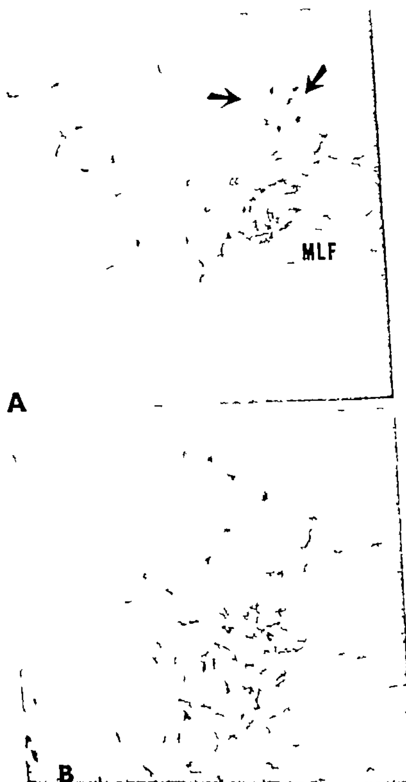


Fig 22 (A) Medium power view through the oculomotor nucleus of Cat 84. Degenerating fibers are shown coursing from the lateral part of the MLF and terminating in the ventral lateral part of the nucleus but not in the dorso-lateral group (*arrows*). Less prominent degeneration in the contralateral nucleus is not visible at this power (B) This high power view of the ipsilateral oculomotor nucleus shown in (A) more clearly shows the localization of degeneration in the ventro-lateral subdivision and not in the dorso-lateral group.

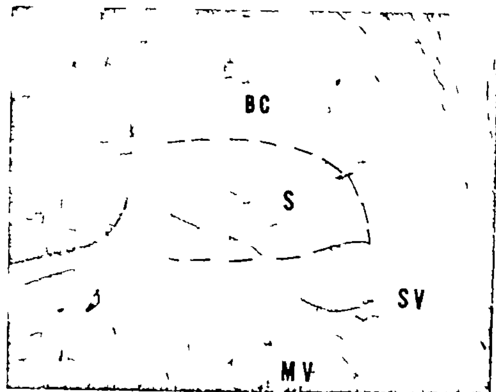


Fig. 0 Section through the rostral half of the superior vestibular nuclei (*S*) of Cat 84 demonstrate no involvement by the lesion. *MV* = motor nucleus of trigeminal nerve. *SV* sensory nucleus of trigeminal nerve.



Fig. 1 High power view through the ipsilateral MLF and trochlear (*4*) nucleus of Cat 84. Degenerated fibers (between arrows) occupy the lateral half of the MLF and there is terminal degeneration in the trochlear nucleus.

plex were strikingly free of any degeneration (Figs. 2? 23).

The degenerating fibers in the ipsilateral MLF could be finally followed to the level of the nucleus of Darkschewitsch and the interstitial nucleus of Cajal where definite preterminal degeneration was found in both nuclei of the ipsilateral side (Fig. 24). No more rostral continuation of degenerating fibers was seen. No degeneration was found in the main nuclear mass of the abducens (VI) nerve nor were any descending fibers seen in the MLF.

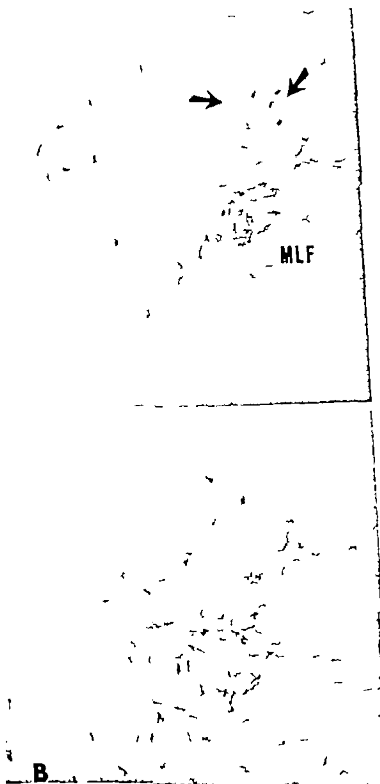


Fig. 22. (A) Medium power view through the oculomotor nucleus of Cat 84. Degenerating fibers are shown coursing from the lateral part of the MLF and terminating in the *ventral lateral part of the* nucleus but not in the dorso-lateral group (*arrows*). Less prominent degeneration in the contralateral nucleus is not visible at this power. (B) This high power view of the ipsilateral oculomotor nucleus shown in (A) more clearly shows the localization of degeneration in the ventrolateral subdivision and not in the dorso-lateral group.



Fig. 23. High power view of the oculomotor complex in Cat 84 showing a degenerated fiber (*arrow*) crossing the midline within nucleus to contralateral side.

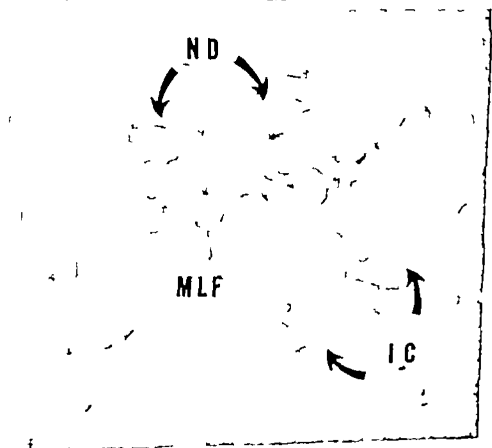


Fig. 24. High power view of terminal degeneration (*arrows*) in the ipsilateral nucleus of Darkschewitsch (*ND*) and interstitial nucleus of Cajal (*IC*) of Cat 84.

Cat no. 94 (see Fig 25)

The lesion essentially followed the same path as in Animal 84 but extended to the rostral pole of the superior vestibular nucleus. In addition to damaging some of the same cells in the nucleus as in the previous animal, this narrow lesion also involved cells in the dorsal portion of the rostral half of the SVN (Fig. 26).

Again degenerating axons were seen emanating from the rostro-medial pole of the SVN (Fig. 27), and entered the ispilateral MLF where they occupied the lateral half of the bundle throughout its ascending course (Figs. 28 and 29).

Terminal degeneration was found in the ipsilateral IV nucleus (Fig. 28 *A* *B*) and in the III complex, the bilateral degeneration pattern was similar to Animal 84, with the ipsilateral side having the more significant amount Notable differences from the previous animal, however were: (1) a small but definite amount of degeneration in the ipsilateral medial cell column, and (2) heavier degeneration in the ventral portion of the lateral cell column in the III complex. These differences would indicate that the rostral part of SVN have projections to the muscles innervated by these cell groups namely the

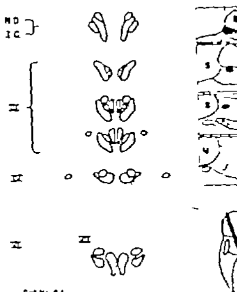


Fig. 25

superior rectus and the medial rectu dorsal lateral cell group in the ocul complex was again strikingly free of a generating fibers (Fig. 30).

The rostral terminal degeneration o degenerating fibers in the MLF aga found in the nucleus of Darkschewits the interstitial nucleus of Cajal.

No degeneration was found in the ab (VI) nucleus of either side.



Fig. [illegible]*3* High power view of the oculomotor complex in Cat 84 showing a degenerated fiber (*arrow*) crossing the midline within nucleus to contralateral side

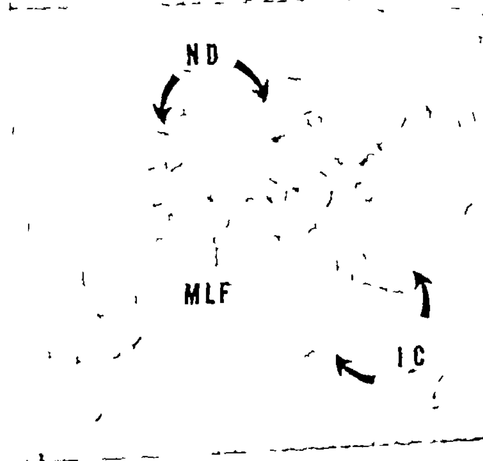


Fig. [illegible]*4* High power view of terminal degeneration (*arrows*) in the ipsilateral nucleus of Darkschewitsch (*ND*) and interstitial nucleus of Cajal (*IC*) of Cat 84



Fig. 28 (*A*) Ipsilateral MLF and trochlear nucleus of Cat 94 showing degenerated axons in lateral half of MLF (arrow) and terminal degeneration in the nucleus. (*B*) Contralateral MLF and trochlear nucleus in Cat 94 showing complete absence of degeneration.

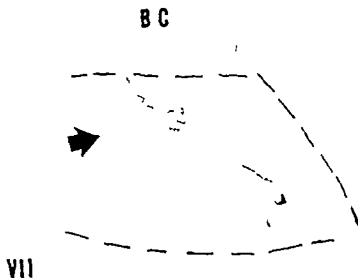


Fig. 26. Arrow points to the lesion in midportion of superior vestibular nucleus (outlined area) of Cat 94.



Fig. 27. High power view of the rostral end of lesion in the superior nucleus of Cat 94. Arrow points to degenerated fiber which will enter the MLF. The brachium conjunctivum (BC) escaped injury.

Cat no. 91 (see Fig 31)

The lesion again involved the dorsal LVN caudal to the SVN. In the SVN the lesion extended from the center of the caudal part of the nucleus to the ventral side of the rostral end of the nucleus. The lesion even extended beyond this into the *brachium conjunctivum*. A large number of degenerating fibers coursing medially in the rostral portion of the *SVN* (Fig. 32) entered the lateral wing of the ipsilateral MLF and retained this characteristic position through the midbrain (Figs. 33, 34).

The terminal degeneration pattern in IV (Fig. 33 *A*) and III was identical to that of Animal 94 except that the amount in the ipsilateral medial cell column of III was heavier (Fig. 35). Terminal degeneration ipsilaterally in the rostral nuclei of Darkschewitsch and Cajal was again seen and no degeneration was found in the VI nucleus.

The heavy terminal degeneration in the *contralateral medial cell group of* III (Fig. 35) and the dorsal part of contralateral IV (Fig. 33 *B*) were associated with the injury to the ipsilateral brachium conjunctivum and not with the SVN lesion.

The lesion in this animal confirmed the findings in Animal 94 that the rostral part of the SVN projected to the ipsilateral medial column and the ventral lateral column of III because this lesion, which interrupted more neurons in the rostral SVN than in Cat 94 was correlated with a heavier degeneration pattern in these two cell divisions of III. The importance of careful reconstruction of the lesion and of the degeneration from control lesions of the brachium conjunctivum was emphasized in this and the following animal.

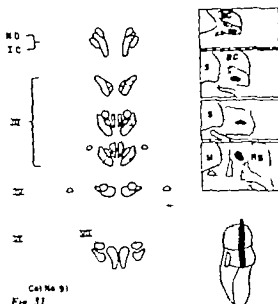


Fig 31



Fig 32 High power view of the ostral end of the lesion (N) in superior vestibular nucleus of Cat 91. Arrow points to degenerated axons leaving the nucleus for the MLF. MV = motor nucleus of trigeminal.

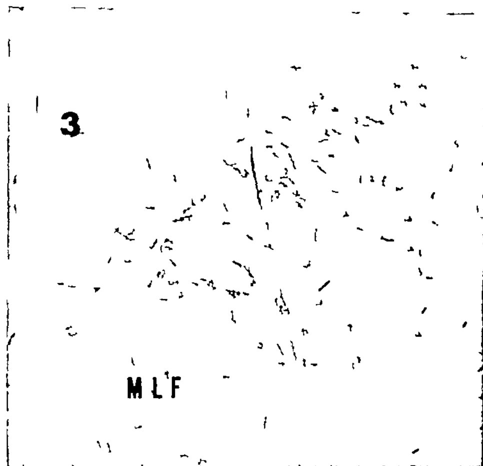


Fig. 29. High power view of the ipsilateral MLF and oculomotor nucleus *(3)* in Cat 94. Degenerated fibers remain located in the lateral part of the MLF and send terminating collaterals to the ventrolateral groups in the oculomotor nucleus.

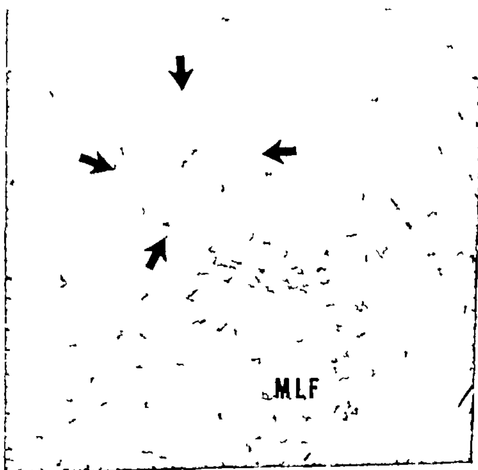


Fig. 30. Ipsilateral oculomotor nucleu of Cat 94 showing absence of degeneration in the dorso-lateral cell group (ar o) while more ventral area are covered with degenerating fibers.

Cat no. 91 (see Fig. 31)

The lesion again involved the dorsal LVN caudal to the SVN. In the SVN the lesion extended from the center of the caudal part of the nucleus to the ventral side of the rostral end of the nucleus. The lesion even extended beyond this into the brachium conjunctivum. A large number of degenerating fibers coursing medially in the rostral portion of the SVN (Fig. 32) entered the lateral wing of the ipsilateral MLF and retained this characteristic position through the midbrain (Figs. 33–34).

The terminal degeneration pattern in IV (Fig. 33 A) and III was identical to that of Animal 94 except that the amount in the ipsilateral medial cell column of III was heavier (Fig. 35). Terminal degeneration ipsilaterally in the rostral nuclei of Darkschewitsch and Cajal was again seen and no degeneration was found in the VI nucleus.

The heavy terminal degeneration in the contralateral medial cell group of III (Fig. 35) and the dorsal part of contralateral IV (fig. 33 B) were associated with the injury to the ipsilateral brachium conjunctivum and not with the SVN lesion.

The lesion in this animal confirmed the findings in Animal 94 that the rostral part of the SVN projected to the ipsilateral medial column and the ventral lateral column of III because this lesion, which interrupted more neurons in the rostral SVN than in Cat 94 was correlated with a heavier degeneration pattern in these two cell divisions of III. The importance of careful reconstruction of the lesion and of the degeneration from control lesions of the brachium conjunctivum was emphasized in this and the following animal.

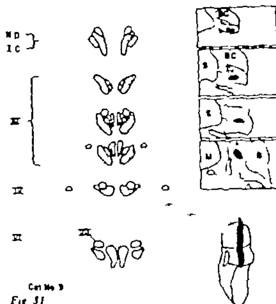


Fig. 31

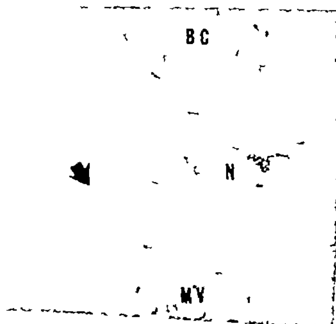


Fig. 3–. High power view of the rostral end of the lesion (N) in superior vestibular nucleus of Cat 91. Arrow points to degenerated axons leaving the nucleus for the MLF. MV = motor nucleus of trigeminal.

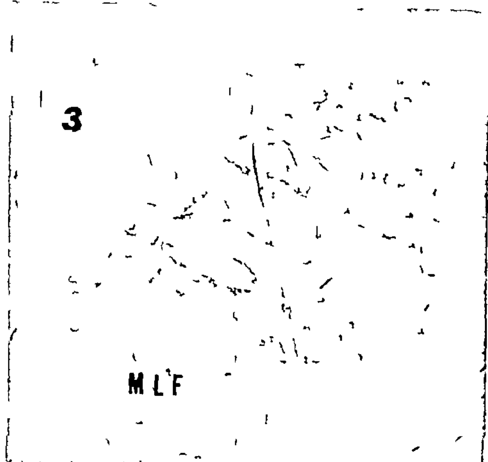


Fig 29 High power view of the ipsilateral MLF and oculomotor nucleus (*3*) in Cat 94. Degenerated fibers remain located in the lateral part of the MLF and send *terminating collaterals to the ventrolateral groups* in the oculomotor nucleus.

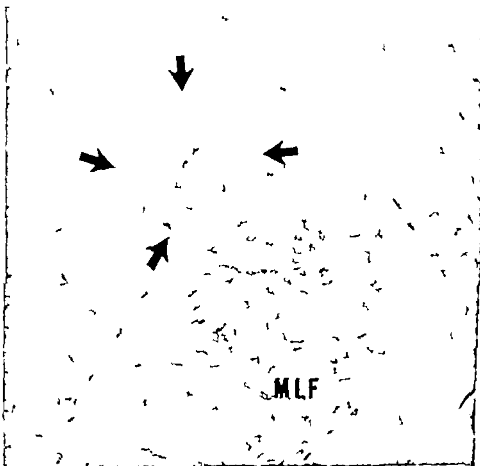


Fig 30 Ipsilateral oculomotor nucleus of Cat 94 showing absence of degeneration in the dorso-lateral cell group (*arrows*) while more ventral areas are covered with degenerating fibers.

Cat no. 91 (see Fig 31)

The lesion again involved the dorsal LVN caudal to the SVN In the SVN the lesion extended from the center of the caudal part of the nucleus to the ventral side of the rostral end of the nucleus. The lesion even extended beyond this into the brachium conjunctivum. A large number of degenerating fibers coursing medially in the rostral portion of the SVN (Fig. 32) entered the lateral wing of the ipsilateral MLF and retained this characteristic position through the midbrain (Figs. 33 34).

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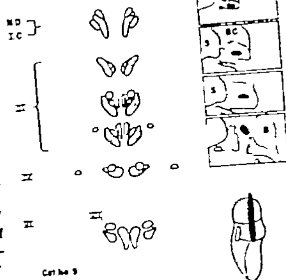


Fig 31

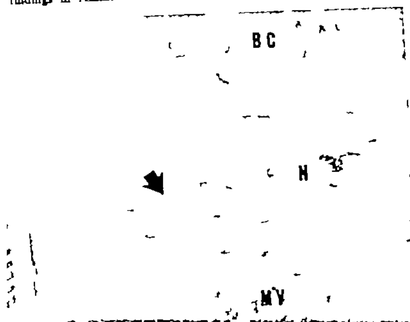


Fig 32 High power view of the rostral end of the lesion (N) in superior vestibular nucleus of Cat 91 Arrow points to degenerated axons leaving the nucleus for the MLF *MV* = motor nucleus of trigeminal.

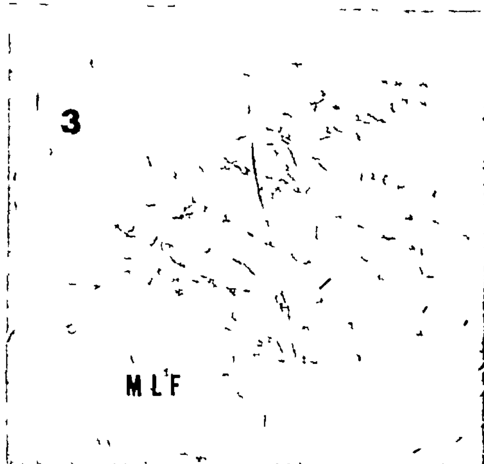


Fig 29 High power view of the ipsilateral MLF and *oculomotor nucleu (3)* in Cat 94 Degenerated fibers remain located in the lateral part of the MLF and send terminating collaterals to the ventrolateral groups in the oculomotor nucleus.

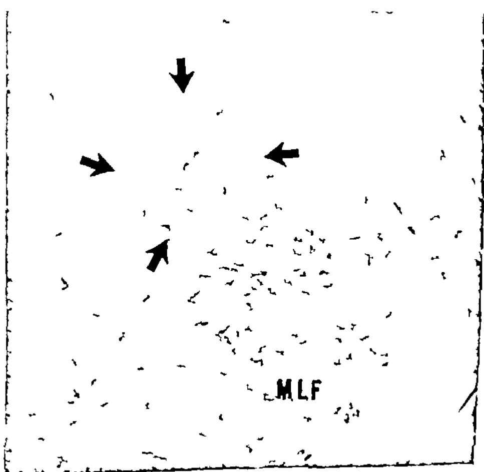


Fig 30 Ipsilater l oculomotor nucleu of C t 94 showing absence of degeneration in the dorso-lateral cell group *(arro)* while more ventral areas are covered with *degenerating fi*bers.

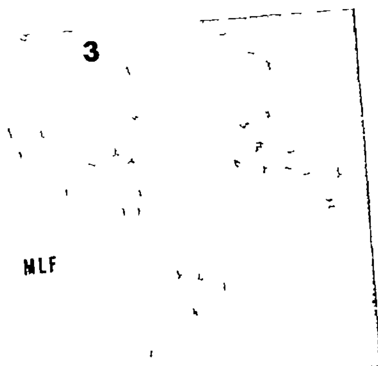


Fig 3A High power view of MLF at level of the caudal oculomotor nucleus (3) of Cat 91. Note large number of degenerated fibers in MLF

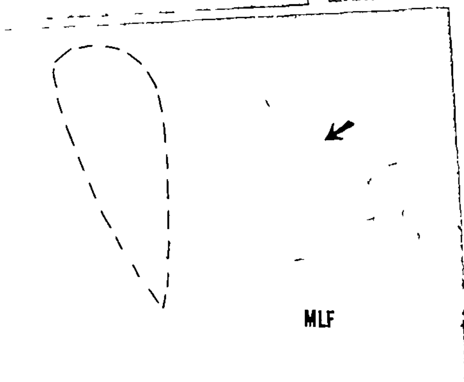


Fig 3 Cat 91 view through the middle of oculomotor nucleus showing terminal degeneration in the ipsilateral medial column and ventrolateral column from the MLF. Other large numbers of degenerating axons are seen laterally. Arrow points to collaterals coursing to the ipsilateral medial cell group. Outlined area indicates terminal degeneration in contralateral medial cell column from injury to brachium conjunctivum.

4

A



4

B

Fig 11 (A) Ipsilateral MLF and t chlear (4) nu- leus of Cat 91 showing the large mount of degen- erat on l calized t lateral pa t of MLF (B) Contra lateral MLF and t chlear nucleus of Cat 91 No de- generat n MLF or main part of t chle ucle s Only a few degener ted te minal t) re f und lo g dorsal r m of the nucle (f Fig 1

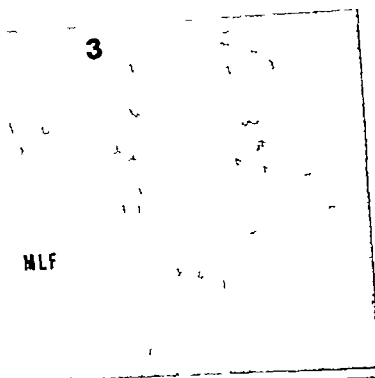


Fig 34 High power view of MLF at level of the caudal oculomotor nucleus (*3*) of Cat 91. Note large number of degenerated fibers in MLF

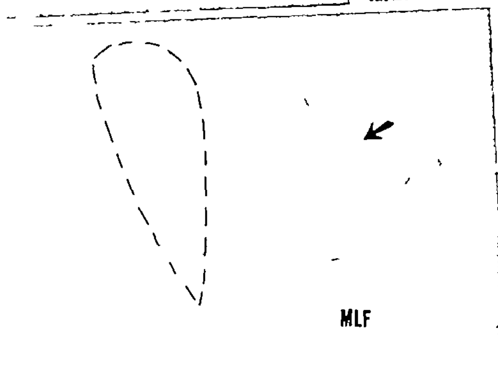


Fig 35 Cat 91 view through the middle of oculomotor nucleus showing terminal degeneration in the ipsilateral medial column and ventrolateral column from the MLF here large numbers of degenerating axons are seen laterally. Arrow points to collaterals coursing to the ipsilateral medial cell group. Outlined area indicates terminal degeneration in contralateral medial cell column from injury to brachium conjunctivum.

4

A

4

B

Fig. 33. (*A*) Ipsilateral MLF and trochlear (*4*) nuclei of Cat 91 showing the large amount of degeneration localized to lateral part of MLF. (*B*) Contralateral MLF and trochlear nucleus of Cat 91. No degeneration in MLF or main part of trochlear nucleus. Only a few degenerated terminal (*m*) are found along dorsal rim of the nucleus. Cf. Fig. 17.

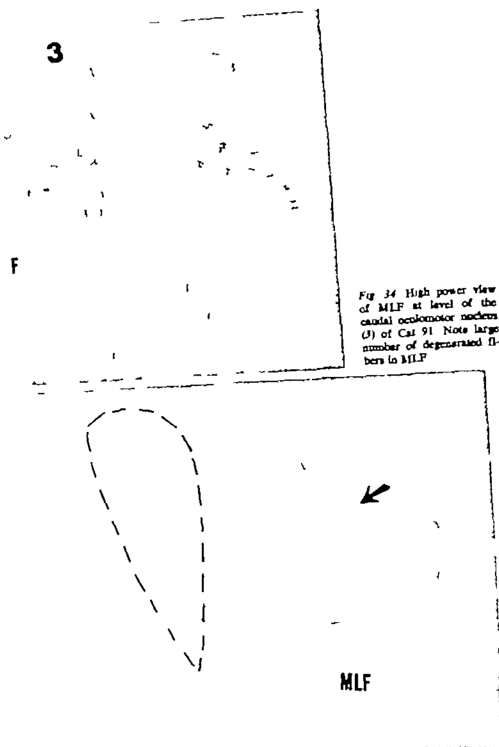


Fig 34 High power view of MLF at level of the caudal oculomotor nucleus (3) of Cat 91 Note large number of degenerated fibers in MLF

Cat 91 ...ew through the middle of oculo... nucleus showing terminal degeneration in the ...eral medial column and ventrolateral column ... the MLF here large numbers of degenerating ... are seen laterally Arrow points to collaterals coursing to the ipsilateral medial cell group. Outlined area indicates terminal degeneration in contralateral medial cell column from injury to brachium conjunctivum.

Cat no. 66 (see Fig 36)

This very small lesion involved the dorsal LVN but was located in the lateral portion of the SVN throughout most of its extent (Fig 37) The rostral pole of the SVN was spared as the lesion was more dorsally positioned and eventually extended into the brachium conjunctivum.

A very few degenerating fibers were followed up the MLF to the IV (Fig 38) and III nuclei and the two rostral nuclei of the ipsilateral side Noteworthy was the absence of degeneration in the ipsilateral medial cell column of III (Fig. 39) this again correlated with the intact rostral portion of the SVN

The most striking degeneration pattern was that associated with the lesion in the brachium conjunctivum and involved the dorsal part of the contralateral IV nucleus (Fig 38 *B*) and the medial cell column of contralateral III (Fig. 39 *A*)

The most important point to be derived from this lesion was that it suggests that the smaller cells that make up the lateral portion of SVN probably do not contribute to the ascending ocular projection This lesion should have injured a significant number of these cells by its location. The very small number of ipsilateral degenerating fibers probably belong to a few of the larger cells which may be found interspersed with these small ones in the periphery of the SVN

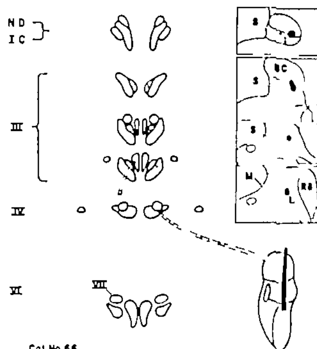


Fig 36

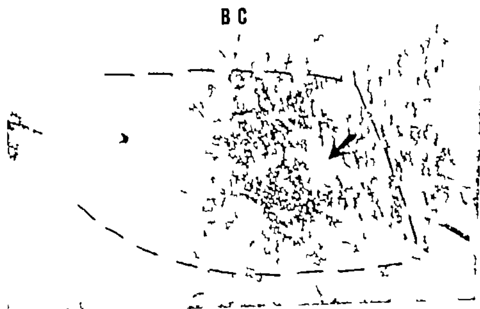


Fig 37 Transverse section through the superior vestibular nucleus (outlined area) of Cat 66. Arrow points to the small diameter lesion in the lateral portion of the nucleus.

Summary of lesions in SVN

Lesions and degeneration patterns indicated that the superior vestibular nucleus projected only into the ipsilateral MLF where its fibers are located in the lateral half. These fibers terminated in the IV and III nuclei in the following manner: ipsilateral IV, bilateral intermediate and ventral lateral cell groups of III with ipsilateral side more marked, ipsilateral medial cell group of III.

Neurons in the caudal-medial part of the SVN project primarily to IV (contralateral superior oblique) and the intermediate lateral cell group of III (ipsilateral inferior oblique). Those in the rostral and central part of SVN project mainly to IV and to the medial cell column (contralateral superior rectus) and ventral-lateral group (ipsilateral medial rectus) of III.

The SVN does not project to the VI nucleus or to the dorsal-lateral cell group of III (innervates inferior rectus). This ascending projection terminates rostrally in the nucleus of Darkschewitsch and the interstitial nucleus of Cajal.

II. Lesions in the medial vestibular nucleus and ventral lateral vestibular nucleus

The second group of lesions which produced ascending degeneration from the vestibular nuclei were those involving the more caudal parts of the vestibular complex, specifically the medial vestibular nucleus and the adjacent ventral division of the lateral vestibular nucleus (Deiters). Lesions in this area were created by inserting the probe caudal-ventral to the dorsal acoustic stria and medial to the dorsal surface of the restiform body. It was then passed in a rostral and slightly ventral direction to varying depths into the brainstem.

Since the probe initially passed through the descending (inferior) vestibular nucleus, in almost all cases it was necessary to determine whether this nucleus contributed any ascending fibers to the MLF. Several lesions in this nucleus alone produced degenerating fibers which passed medially and entered both MLF. Ipsilaterally the fibers were located in the dorso-lateral aspect of the MLF (Fig. 45), and contralaterally they were located medially in the bundle.

These degenerated fibers were followed in a descending direction in the MLF but none could be followed to upper levels of the MLF or to the nuclei of IV and III. This indicated that the descending vestibular nucleus did not contribute ascending fibers in the MLF. Others (Carpenter, 1966; Buchanan, 1937) have reported identical findings with regard to the descending nucleus. Any ascending degeneration, therefore, may be correlated with the lesion in the medial and lateral vestibular nuclei.

Cat no 66 (see Fig 36)

This very small lesion involved the dorsal LVN but was located in the lateral portion of the SVN throughout most of its extent (Fig. 37) The rostral pole of the SVN was spared as the lesion was more dorsally positioned and eventually extended into the brachium con junctivum.

A very few degenerating fibers were followed up the MLF to the IV (Fig. 38) and III nuclei and the two rostral nuclei of the ipsilateral side. Noteworthy was the absence of degeneration in the ipsilateral medial cell column of III (Fig 39) thus again correlated with the intact rostral portion of the SVN

The most striking degeneration pattern was that associated with the lesion in the brachium conjunctivum and involved the dorsal part of the contralateral IV nucleus (Fig 38 *B*) and the medial cell column of contralateral III (Fig 39 *A*)

The most important point to be derived from this lesion was that it suggests that the smaller cells that make up the lateral portion of SVN probably do not contribute to the ascending ocular projection This lesion should have injured a significant number [of] these cells by its location The very s[mall] number of ipsilateral degenerating fibers pr[ob]ably belong to a few of the larger cells wh[ich] may be found interspersed with these s[mall] ones in the periphery of the SVN

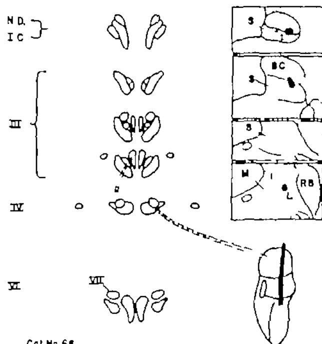


Fig 36

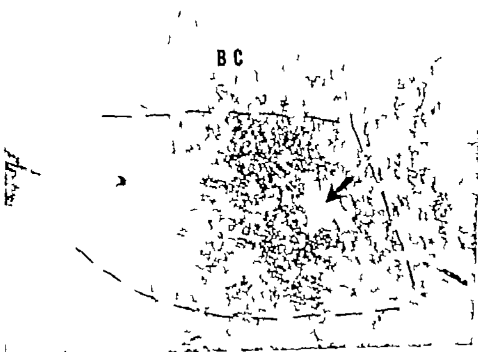


Fig 37 Transverse sect[ion] through the superior v[es]tibular nucleus (outl[ined] area) of Cat 66 Arr[ow] points to the small diam[e]ter lesion in the late[ral] portion of the nucleus

Summary of lesions in SVN

Lesions and degeneration patterns indicated that the superior vestibular nucleus projected only into the ipsilateral MLF where its fibers are located in the lateral half. These fibers terminated in the IV and III nuclei in the following manner: ipsilateral IV bilateral intermediate and ventral lateral cell groups of III with ipsilateral side more marked, ipsilateral medial cell group of III.

Neurons in the caudal-medial part of the SVN project primarily to IV (contralateral superior oblique) and the intermediate lateral cell group of III (ipsilateral inferior oblique). Those in the rostral and central part of SVN project mainly to IV and to the medial cell column (contralateral superior rectus) and ventral-lateral group (ipsilateral medial rectus) of III.

The SVN does not project to the VI nucleus or to the dorsal-lateral cell group of III (innervates inferior rectus). This ascending projection terminates rostrally in the nucleus of Darkschewitsch and the interstitial nucleus of Cajal.

II. *Lesions in the medial vestibular nucleus and ventral lateral vestibular nucleus*

The second group of lesions which produced ascending degeneration from the vestibular nuclei were those involving the more caudal parts of the vestibular complex, specifically the medial vestibular nucleus and the adjacent ventral division of the lateral vestibular nucleus (Deiters). Lesions in this area were created by inserting the probe caudal-ventral to the dorsal acoustic stria and medial to the dorsal surface of the restiform body. It was then passed in a rostral and slightly ventral direction to varying depths into the brainstem.

Since the probe initially passed through the descending (inferior) vestibular nucleus, in almost all cases it was necessary to determine whether this nucleus contributed any ascending fibers to the MLF. Several lesions in this nucleus alone produced degenerating fibers which passed medially and entered both MLF. Ipsilaterally the fibers were located in the dorso-lateral aspect of the MLF (Fig. 45), and contralaterally they were located medially in the bundle.

These degenerated fibers were followed in a descending direction in the MLF but none could be followed to upper levels of the MLF or to the nuclei of IV and III. This indicated that the descending vestibular nucleus did not contribute ascending fibers in the MLF. Others (Carpenter 1966; Buchanan, 1937) have reported identical findings with regard to the descending nucleus. Any ascending degeneration, therefore, may be correlated with the lesion in the medial and lateral vestibular nuclei.

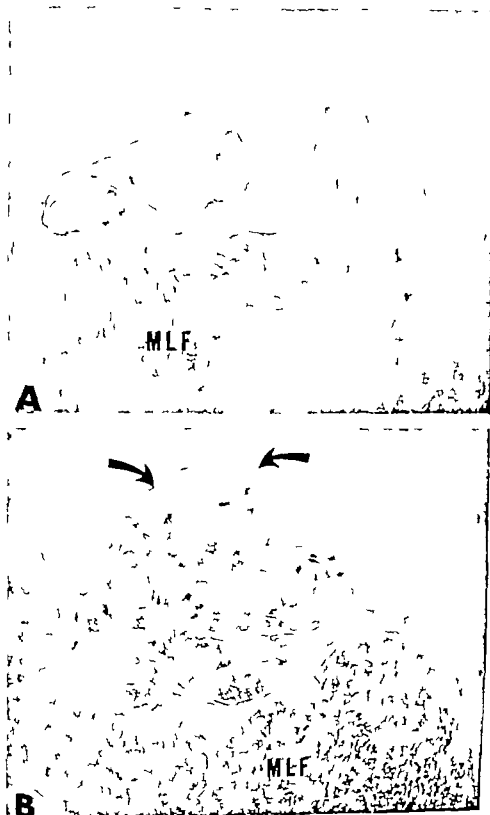


Fig 38 (*A*) Ipsilateral MLF and trochlear nucleus of Cat 66. There is a small amount of terminal degeneration scattered throughout the nucleus. (*B*) Contralateral MLF and trochlear nucleus of Cat 66. Arrows point to a minimal amount of degeneration along dorsal aspect of the nucleus resulting from injury to brachium co junctivum. Cf F g. 17

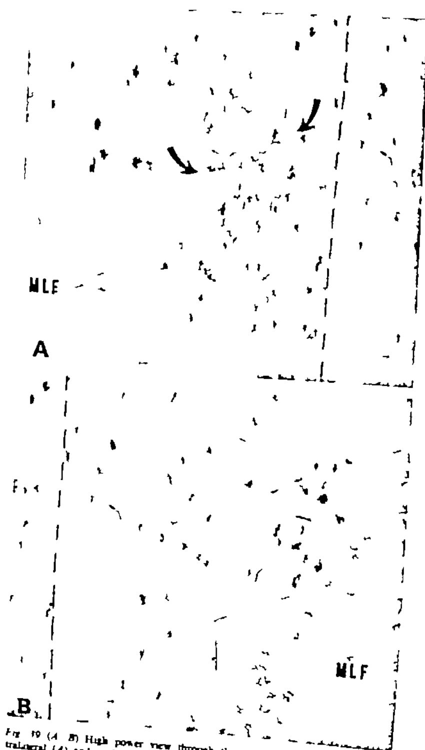


Fig. 19 (*A*, *B*) High power view through the contralateral (*A*) and ipsilateral (*B*) oculomotor nucleus of Cat 66. There is significant degeneration in the contralateral medial cell column (*arrows*) but practically none in the ipsilateral nucleus. Broken vertical line designates the midline.

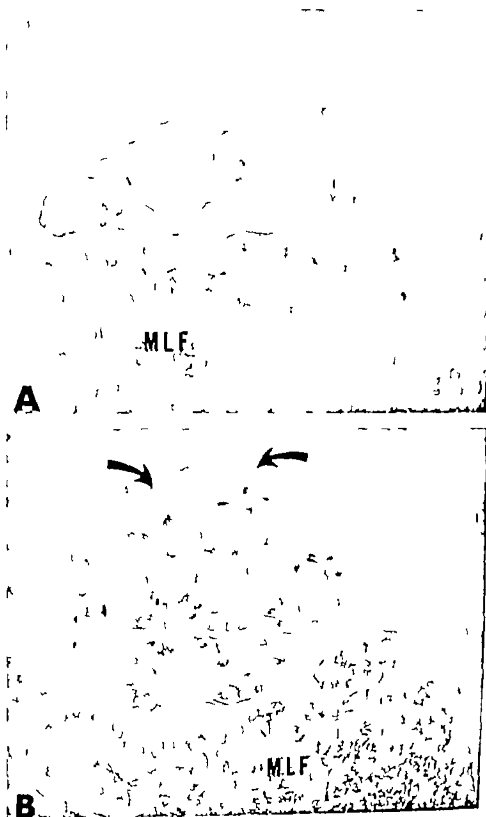


Fig 38 (*A*) Ipsilateral MLF and trochlear nucleus of Cat 66. There is a small amount of terminal degeneration scattered throughout the nucleus. (*B*) Contralateral MLF and trochlear nucleus of Cat 66. Arrows point to a minimal amount of degeneration along dorsal aspect of the nucleus resulting from injury to brachium conjunctivum Cf Fig. 17

Cat no. 98. (Fig. 41)

The lesion in this animal involved a minimal part of the DVN but passed through the lateral most portion of the MVN (Fig. 42) and finally the ventral division of LVN to its rostral pole without injuring the SVN. The olivocochlear bundle was interrupted as it coursed through the ventral LVN (Fig. 43).

Two tracts of degenerating fibers were identified in an ascending direction from the lesion. (1) The main mass of degenerating axons passed directly medially from the MVN lesion and adjacent LVN. These fibers characteristically passed ventral to the facial genu toward the ipsilateral VI nucleus where profuse terminal degeneration was seen (Fig. 44). Degenerating axons destined for the MLF continued past the VI nucleus and either positioned themselves in the dorso-lateral corner of the ipsilateral MLF (Fig. 45) or crossed the midline to assume a dorso-medial location in the contralateral MLF (Fig. 46). The degenerating fibers in the ipsilateral MLF represent descending fiber projections from the DVN and MVN.

The contralateral degeneration in the MLF could be followed to both higher and lower levels of the brainstem and, therefore, probably represent bifurcating axons. The contralateral ascending degenerating fibers retained a medial position in the MLF and terminated in the contralateral IV nucleus (Fig. 47 *A, B*) and the contralateral III nucleus where all the subgroups, except the dorso-lateral one, were found covered with degenerating terminals. Degenerating collaterals crossing the midline within the III nucleus to similar ipsilateral subgroups were again found. Finally these degenerating fibers in the contralateral MLF terminated in the nuclei of Darkschewitsch and Cajal of that side. (2) A number of ipsilateral ascending degenerating fibers were seen as scattered fragmented axons positioned lateral to the ipsilateral MLF (Fig. 48) and arising from the rostral end of the lesion in the ventral LVN. These fibers did not form connections with the ipsilateral IV nucleus but arched medially over the MLF at the level of the III nucleus to terminate only in the dorso-lateral cell group which innervates the inferior rectus muscle (Fig. 49). No degeneration was found in the nuclei of Darkschewitsch or Cajal of this side.

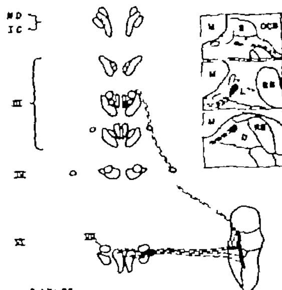


Fig. 41

Cat no. 96 (see Fig 40)

The lesion after passing through the most medial aspect of the DVN extended into the lateral most portion of the medial vestibular nucleus where it adjoins the ventral aspect of the LVN The most rostral extent of the lesion involved a very small part of the caudal end of the ventral LVN Since the DVN does not contribute any ascending fibers to the MLF the degeneration in this bundle can be considered to be from the MVN

Degenerating fibers passed directly medially from the lesion and coursed under the genu of the facial nerve. Some fibers at this point terminated in the ipsilateral VIth nucleus either by collaterals or by termination of a parent fiber The majority of the fibers passed through or above the MLF to become located in the dorso-medial aspect of the contralateral MLF and the lateral aspect of the ipsilateral MLF

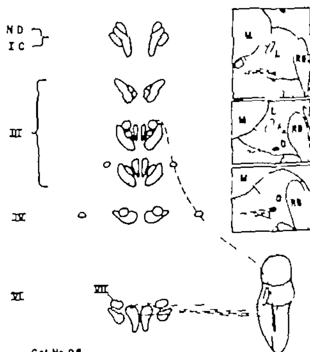


Fig 40

Only the degenerating fibers in the contralateral MLF could be found in higher levels of the MLF and therefore represented the ascending degeneration from this lesion. They characteristically occupied a more medial position in the MLF than the fibers from the SVN Preterminal degeneration was found in the contralateral IV and III nuclei Degeneration in the contralateral oculomotor complex involved the intermediate and ventral-lateral cell groups while the dorsal-lateral group and the medial cell column were completely free However a small but definite amount of fine degenerating preterminals was found ipsilaterally in only the dorsal lateral cell group of III. These fibers did not arise from the ipsilateral MLF but from a pathway outside and lateral to this bundle. A few degenerating fibers were seen in this area and followed caudally to the region of the lesion

The most rostral preterminal degeneration was seen in the contralateral nucleus of Darkschewitsch and the interstitial nucleus of Cajal and represented the rostral termination of the degenerating fibers in the contralateral MLF

Cat no. 98 (Fig. 41)

The lesion in this animal involved a minimal part of the DVN but passed through the lateral most portion of the MVN (Fig. 42) and finally the ventral division of LVN to its rostral pole without injuring the SVN. The olivocochlear bundle was interrupted as it coursed through the ventral LVN (Fig. 43).

Two tracts of degenerating fibers were identified in an ascending direction from the lesion. (1) The main mass of degenerating axons passed directly medially from the MVN lesion and adjacent LVN. These fibers characteristically passed ventral to the facial genu toward the ipsilateral VI nucleus where profuse terminal degeneration was seen (Fig. 44). Degenerating axons destined for the MLF continued past the VI nucleus and either positioned themselves in the dorso-lateral corner of the ipsilateral MLF (Fig. 45) or crossed the midline to assume a dorso-medial location in the contralateral MLF (Fig. 46). The degenerating fibers in the ipsilateral MLF represent descending fiber projections from the DVN and MVN.

The contralateral degeneration in the MLF could be followed to both higher and lower levels of the brainstem and, therefore, probably represent bifurcating axons. The contralateral ascending degenerating fibers retained a medial position in the MLF and terminated in the contralateral IV nucleus (Fig. 47 *A*, *B*), and the contralateral III nucleus where all the subgroups, except the dorso-lateral one were found covered with degenerating terminals. Degenerating collaterals crossing the midline within the III nucleus to similar ipsilateral subgroups were again found. Finally these degenerating fibers in the contralateral MLF terminated in the nuclei of Darkschewitsch and Cajal of that side. (2) A number of ipsilateral ascending degenerating fibers were seen as scattered fragmented axons positioned lateral to the ipsilateral MLF (Fig. 48) and arising from the rostral end of the lesion in the ventral LVN. These fibers did not form connections with the ipsilateral IV nucleus but arched medially over the MLF at the level of the III nucleus to terminate only in the dorso-lateral cell group which innervates the inferior rectus muscle (Fig. 49). No degeneration was found in the nuclei of Darkschewitsch or Cajal of this side.

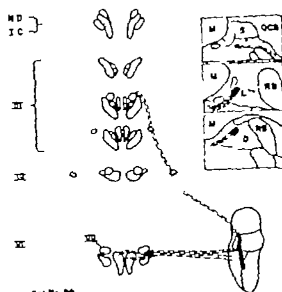


Fig. 41

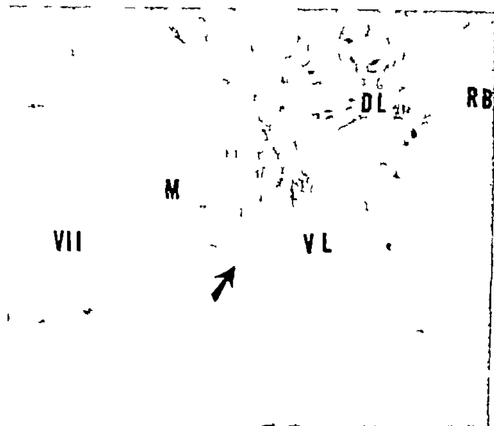


Fig 42 Low power view of the lesion (*arrow*) involving the ventral division of the lateral vestibular nucleus (*VL*) and the adjacent medial vestibular nucleus (*M*) in Cat 98. Cf. Figs. 4 and 5 *DL* = Dorsal division of the lateral vestibular nucleus.

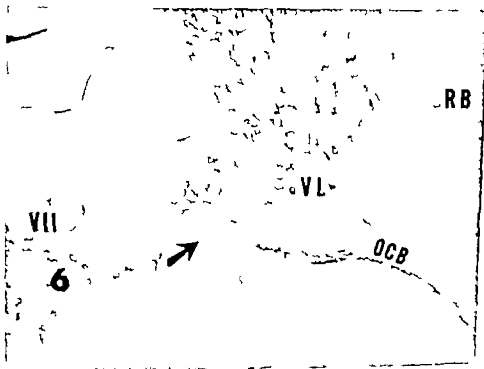


Fig 43 Rostral end of the lesion (*arrow*) in Cat 98. The olivocochlear bundle (*OCB*) was injured as it courses through the ventral lateral vestibular nucleus (*VL*) and is degenerated The abducens nucleus (*6*) is shown at higher magnification in Fig. 44



Fig 44 High power iew of ipsilateral abducens nucleus in preceding photomicrograph. Many degenerating fibers are seen coming from the lesion at right and terminating around neurons of the nucleus (*arrow*). Others continue toward the MLF as shown in next figure. *VII* = Facial nerve germ.

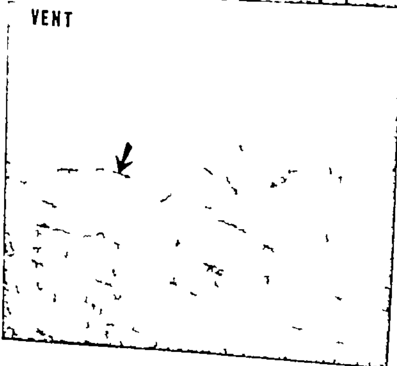


Fig 45 High power view of the ipsilateral MLF in Cat 98 Note how degenerated fibers are localized in the dorso-lateral corner of this MLF (black dots at right center of figure). These are descending fibers and will not be isable at more rostral levels. Degenerating fibers that form the contralateral ascending ystem, pass through the ipsilateral MLF (*arrow*) toward the midline at left of figure. *VENT* = Fourth ventricle.

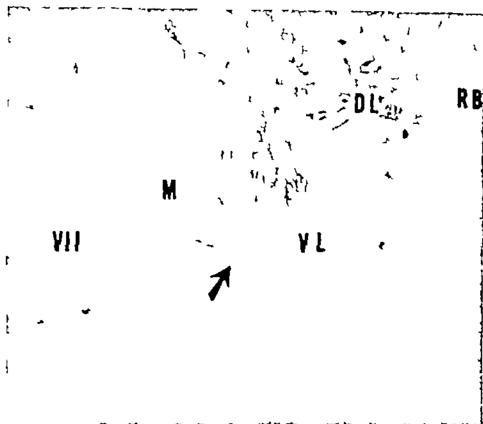


Fig 4 Low power view of the lesion (*arrow*) involving the ventral division of the lateral vestibular nucleus (*VL*) and the adjacent medial vestibular nucleus (*M*) in Cat 98 Cf Figs. 4 and 5 *DL* – Dorsal division of the lateral vestibular nucleus.

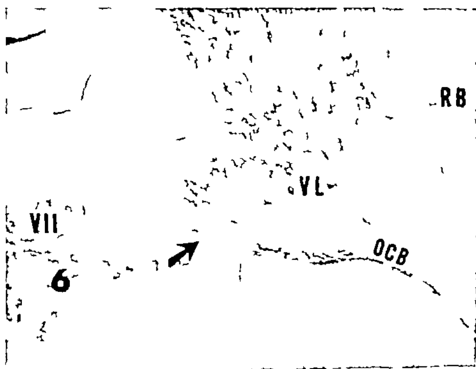


Fig 43 Rostral end of the lesion (*arrow*) in Cat 98 The olivocochlear bundle (*OCB*) was injured as it courses through the ventral lateral vestibular nucleus (*VL*) and is degenerated. The abducens nucleus (*6*) is shown at higher magnification in Fig. 44



Fig. 44 High power view of ipsilateral abducens nucleus in preceding photomicrograph. Many degenerating fibers are seen coming from the lesion at right and terminating around neurons of the nucleus (*arrow*). Others continue toward the MLF as shown in next figure. *VII* = Facial nerve genu.



Fig 45 High power iew of the ipsilateral MLF in Cat 98 Note how degenerated fibers are localized in the dorso-lateral corner of this MLF (black dots at right center f figure). These are descending fibers and will not be visible at more rostral levels. Degenerating fibers that form the contralateral ascending system, pass through the ipsilateral MLF (*arrow*) toward the midline at left f figure. *VENT* = Fourth entricle.

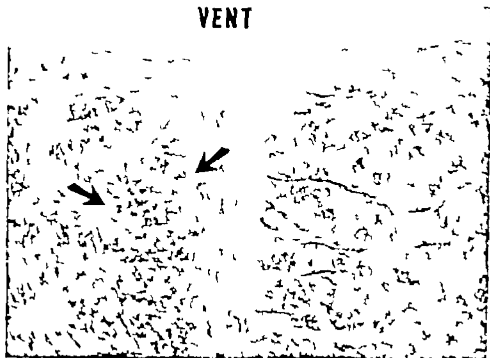


Fig. 46. High power view of both MLFs in Cat 98. Level is slightly more rostral than in preceding figure. The zone of black dots between arrows represent degenerating axons in the dorso-medial aspect of the contralateral MLF.

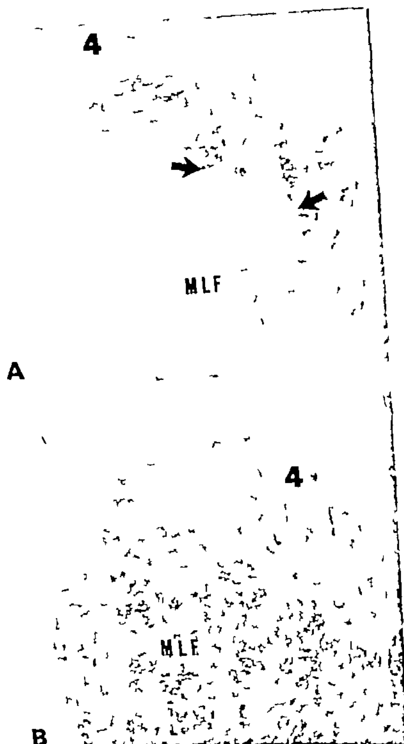


Fig. 47. (A, B) High power view of the contralateral (A) and ipsilateral (B) MLF and trochlear nuclei in Cat 98. Arrows point to the degenerating ascending fibers in the medial part of contralateral MLF. There is also terminal degeneration in the contralateral trochlear nucleus (A) while the ipsilateral MLF and trochlear nucleus is free.



Fig 48 High power view of the area lateral to the ipsilateral MLF shown in Fig. 47 *B* Arrows point out an area of scattered degenerated fibers which lie outside the lateral limit of the MLF These fibers represent the ascending tract of Deiters

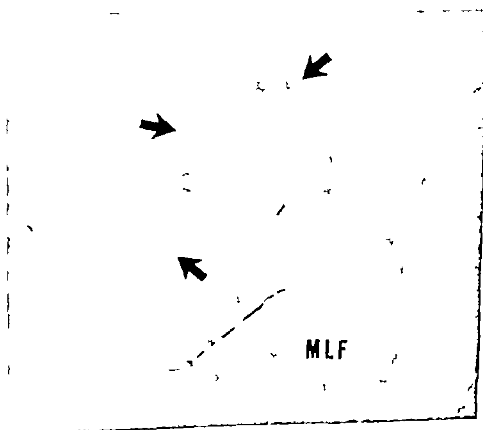


Fig 49 High power view of the ipsilateral oculomotor nucleus of Cat 98. Arrows point out degeneration in the dorso-lateral group which is supplied by the ascending tract of Deiters. Note the absence of degeneration in the nuclear zones to the left and inferior to the dorso-lateral group.

Cat no. 100 (see Fig 50)

The lesion in this animal was similar to that in the previous animal but was larger in diameter and more laterally placed in the ventral lateral vestibular nucleus (Fig. 51).

At the level of the facial genu degenerating fibers were followed medially from the lesion and passed ventral to the facial nerve *to enter the lateral aspect of the ipsilateral* MLF and the dorso-medial corner of the contralateral MLF. The degenerating fibers in the *ipsilateral MLF represent descending* projections from the descending and medial vestibular nuclei while those in the contralateral MLF are the fibers of the ascending system from the medial nucleus. Many of these contralateral degenerating fibers bifurcate and send a descending branch as well in the MLF.

Preterminal degeneration from these degenerating fibers entering the MLF was found mainly in the ipsilateral VI nucleus but also in the contralateral VI nucleus. The degenerating fibers at higher levels of the MLF were found in the medial portion of only the contralateral bundle. Fine terminal and preterminal degeneration was observed in the contralateral IV, III (intermediate and ventral-lateral cell groups, and medial cell group) and the nuclei of Darkschewitsch and Cajal.

The dorsal-lateral cell column of the con-

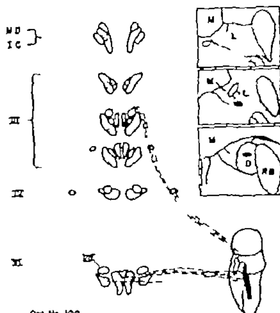


Fig 50

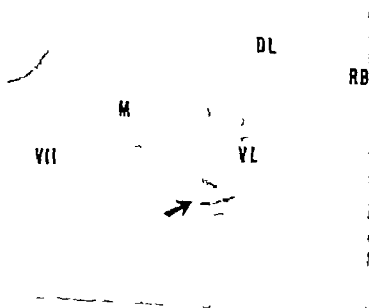


Fig 51 Low power view of the lesion (arrow) in Cat 100. The lesion is similar in location and diameter but not as long (Cf. 50).



Fig 48. High power of the area lateral t ipsilateral MLF show Fig. 47 *B* Arrows out an area of scattere generated fibers whic outside the lateral lin the MLF These fibers resent the ascending of Deiters"

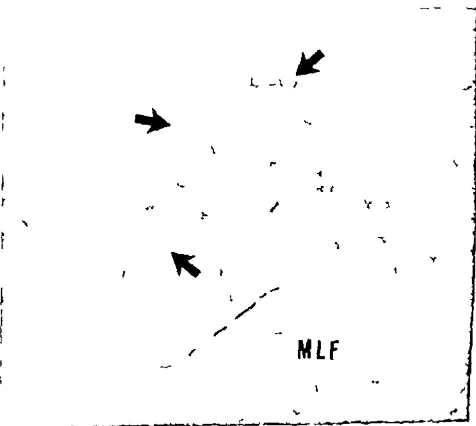


Fig 49 High power of the ipsilateral oculo tor nucleus of Cat *Arrows point out dege ation in the dorso-lat group which is supplied the ascending tract Deiters. Note the abse of degeneration in the* clear zones to the left inferior to the dorso-la al group

tralateral III nucleus was conspicuously free of degeneration while the same subnuclear group on the ipsilateral side was covered with a more significant amount of preterminal degeneration than in cat Number 98 (Fig. 52 *A B*). Degenerating fibers supplying this ipsilateral group were definitely seen ascending from the rostral end of the lesion in the lateral vestibular nucleus to travel ventral to the superior vestibular nucleus and then gradually assumed a position in the brainstem lateral to the MLF until the level of the III nucleus was reached. The fibers then arched over the dorso-lateral aspect of the MLF to terminate in the dorsal-lateral cell group. This tract of degenerating fibers would appear to correspond to the ascending tract of Deiters" of Winkler (1918) Buchanan (1937) and to the vestibulo-tegmentalis lateralis" of Muskens (1913–14).

The lesions in animals 98 and 100 confirmed the course and origin from the MVN of the contralateral ascending ocular projection in the MLF and by involving more of the ventral part of the lateral vestibular nucleus than cat Number 96 clearly demonstrated the source of the degenerating fibers to the ipsilateral dorsal-lateral cell group of the III nucleus. The projection of this area (MVN and LVN) to both VI nuclei but in a predominantly ipsilateral fashion was also demonstrated by this larger lesion.

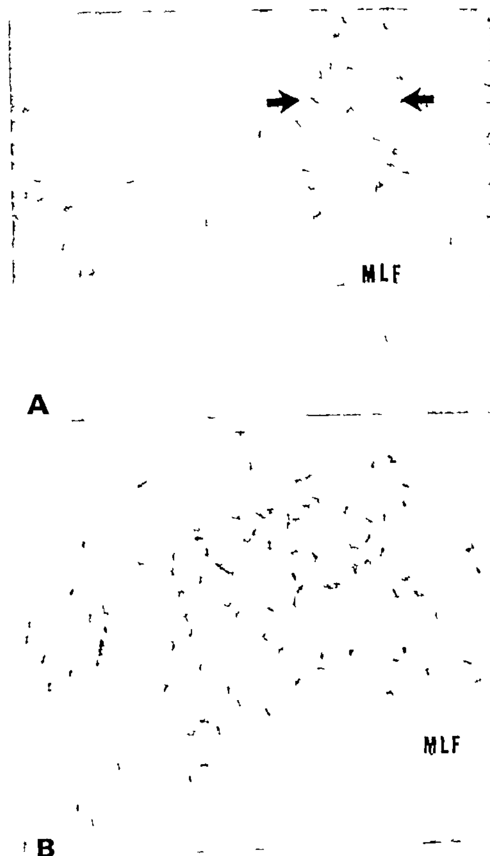


Fig. 52 (A) Low power view of oculomotor nucleus in Cat 100. Arrows mark the pallateral dorsolateral cell group which is covered with terminal degeneration shown to better advantage in next figure. Note also the diffuse degener tion throughout the contralateral (*left*) side of the nucleus. (B) High power view of the area marked by arrow i preceding figure

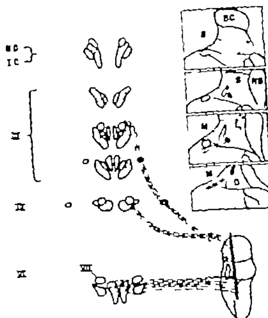


Fig 55

clear complex, and the contralateral nuclei of Darkschewitsch and Cajal.

Lesions in the ventral division of the lateral vestibular nucleus confirmed its descending projection in the ipsilateral vestibulo-spinal tract but also proved that it has connections with the ipsilateral oculomotor (III) nucleus but not to the trochlear (IV) nucleus. The projection to the oculomotor complex was quite unique because it terminated in the only cell group that neither the ipsilateral nor contralateral MLF ascending pathways formed connections with. Since the lateral nucleus (Deiters) projected its ascending fibers in a tract lateral to the MLF it seemed quite appropriate to retain the terminology "ascending tract of Deiters" as used by Winkler (1918) and Buchanan (1937).

Summary of lesions in the medial vestibular nucleus and ventral lateral vestibular nucleus

Lesions in this group of animals indicated that the area of small cells just ventral to the caudal-ventral end of the lateral vestibular nucleus probably represents an extension of the rostral part of the medial vestibular nucleus because its projections are identical to those of the medial vestibular nucleus. These experimental animals indicated that this nucleus sends fibers primarily into the dorsal medial aspect of the contralateral MLF. As these axons pass toward the MLF they make connections with both abducens (VI) nuclei. The fibers that reach the contralateral MLF may send a branch in a descending direction toward the cord levels but for the most part they ascend in the medial portion of the MLF to terminate with the contralateral IV nucleus, the contralateral subdivisions (except for the dorsal-lateral cell group) and to a lesser degree the ipsilateral subgroups of the III nu-

III. *Lesions in the medial lateral and superior vestibular nuclei*

The lesion in Animals 89 (Fig. 55) and 90 (Fig. 54) involved all three of the vestibular nuclei that give ascending projections to the extra-ocular nuclei and, therefore, give a summation of these connections. Both these animals can be discussed together stressing several points.

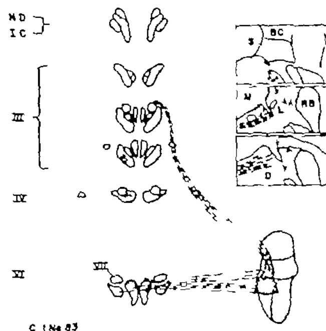
Although the lesion in Animal 90 is larger in diameter (as evidenced by more degeneration from the superior vestibular nucleus), it was more dorsally located in the caudal area, thus injuring cells in the *ventral division of* LVN but barely injured the medial vestibular nucleus while in Animal 89 the more ventral location of the lesion significantly injured this area of MVN. The significant corresponding difference in the degeneration patterns occurred in the contralateral ascending MLF system where the minimal degeneration in the contralateral MLF and the contralateral abducens (VI) nucleus of Animal 90 confirmed that the medial vestibular nucleus and not the lateral vestibular nucleus is mainly responsible for this contralateral projection in the MLF

Cat no. 83 (see Fig 53)

The lesion in this animal was made by a small knife made of a sliver of razor blade. By depressing the knife ventro-laterally through the medial aspect of the dorsal acoustic stria, the entire width of the medial vestibular nucleus and a larger segment of the ventral LVN were involved in the lesion Thus the neurons in the medial portion of the MVN that escaped injury in the previous animals with small, more laterally placed lesions, were now transected. The degeneration pattern was identical to previous animals. However the amount of degeneration in the contralateral ascending system in the MLF was significantly greater indicating that most, if not all, of the rostral half of MVN contributes to the contralateral ascending projection. This further justifies including in the medial vestibular nucleus the area of smaller neurons just ventral to the ventral division of LVN and lateral to the dorsal acoustic stria, rather than in the descending vestibular nucleus (DVN) (Gacek 1969) or lateral vestibular nucleus (LVN) (Fraser 1902, Muskens, 1913–14).

The bilateral but asymmetrical projections to the VI nuclei were also confirmed by this lesion. The ipsilateral nucleus received, by far the greater portion of this projection. It is not possible to precisely say however the degree to which MVN or ventral LVN contribute to these nuclei but the medial nucleus probably gives rise to most of these fiber connections.

Collaterals of the contralateral ascending MLF system that crossed the midline within the III complex were observed as they were in the ipsilateral MLF projection from the superior vestibular nucleus. This finding was also present in the III complex in Animal 98. The degeneration in the "ascending tract of Deiters" which terminated in the ipsilateral dorsal-lateral cell group of III was observed again as in previous animals. The fact that the very rostral end of the lesion undercut the superior vestibular nucleus without damaging it was proved by the absence of any degeneration in the ipsilateral MLF



F g 53

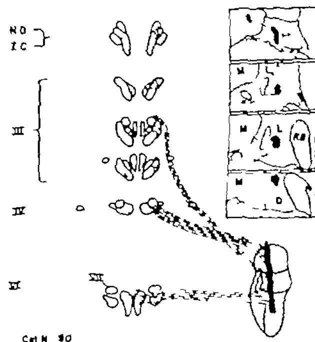


Fig 54

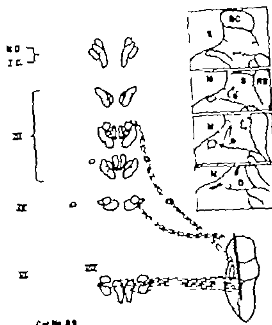


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Fig 56 (A, B) High power views of the ipsilateral (A) and contralateral (B) MLF and trochlear nuclei of Cat 89 showing degeneration of fibers in both ascending systems in the MLF and terminal degeneration in both trochlear nuclei (4)



Fig. 57. High power view of the ipsilateral oculomotor nucleus of Cat 89 showing how degenerated fibers from the ascending tract of Deiters arch over the MLF to terminate in the dorso-lateral group of the nucleus.

Because both ascending MLF systems (fig. 56 *A*, *B*) and the ipsilateral ascending tract of Deiters (Fig. 57) were involved in these animals the degeneration pattern in the oculomotor (III) complex was interesting to examine. All subdivisions of the oculomotor complex except for the contralateral dorsal-lateral cell group (which is innervated by the contralateral lateral vestibular nucleus) were covered with degeneration. This means that all the extraocular muscles of both sides except for the contralateral inferior rectus receive direct innervation from each vestibular nuclear complex or labyrinth.



Fig 56 (*A B*) High power views of the ipsilateral (*A*) and contralateral (*B*) MLF and trochlear nuclei of Cat 89 showing degeneration of fibers in both ascending systems in the MLF and terminal degeneration in both trochlear nuclei (*4*)

this has been designated as descending vestibular nucleus (Gacek 1969) or ventral part of lateral vestibular nucleus (Mutkens, 1913–14) but in terms of its output it should rightfully be included in the medial vestibular nucleus. Studies on the termination of primary vestibular neurons indicated that the descending branches of incoming fibers from the cristae and the maculae terminate in this area to a large degree.

The fibers of this ascending system projected to and terminated in the contralateral trochlear nucleus and the contralateral divisions of the oculomotor complex in a similar fashion to the innervation pattern of the ipsilateral ascending system from the superior vestibular nucleus. The rostral terminus of this system also concerned the interstitial nucleus of Cajal and the nucleus of Darkschewitsch. The contralateral ascending system was also devoid of termination in the cell group (dorso-lateral) of the oculomotor complex which supplies the inferior rectus muscle.

It appears then that the two ascending systems in the MLF arise from the superior and the medial vestibular nuclei and project in a balanced fashion up the two sides of the brain stem to similar areas of termination in the III and IV cranial nerve nuclei. Rostral terminations in the midbrain nuclei of Darkschewitsch and Cajal are also similar.

(b) The third system of ascending fibers projected only to the oculomotor (III) complex and was not located in the MLF. It coursed in a less discrete tract which occupied the lateral part of the brainstem and midbrain. In the midbrain area it traveled just lateral to the lateral wing of the MLF. This tract has been called the "ascending tract of Deiters" in older literature indicating that its origin is from the lateral vestibular nucleus or nucleus of Deiters. Mutkens (1914) who originally described this tract felt that it originated from medium cells in the dorsal division of Deiters while Buchanan (1937) concluded that the ventral portion of Deiters was the source of this system. Previous descriptions, however, have not described precise termination of this tract because of the limitation of the Marchi method to myelinated portions of nerve fibers.

In this study the origin of this tract from the ventral division of the lateral vestibular nucleus was confirmed. This finding would explain Brodal's (1957) description of absence of changes in some cells of the rostro-ventral part of LVN when the vestibulo-spinal tract was sectioned high in the cervical region. The large multipolar neurons in the dorsal and caudal regions of the LVN exhibited characteristic retrograde reaction changes following such a lesion indicating that they contribute to the vestibulo-spinal tract. The unchanged neurons in the ventral division of LVN probably represent those that contribute to the "ascending tract of Deiters".

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Connections to the abducens nuclei appeared to come only from lesions in the mid and caudal areas of the vestibular complex. Ipsilateral and contralateral connections to these nuclei were found from the medial vestibular nucleus (see Cats 89 and 100) and *primarily* ipsilateral connections to the nucleus were found from the descending vestibular nucleus. The descending vestibular nucleus, as far as could be determined from lesions isolated in it, contributed only descending projections in both medial longitudinal fasciculi.

These results can explain the findings of physiological studies such as the one of Tokumasu et al. (1969) which had been reviewed earlier. They found that a stimulating electrode in the superior vestibular nucleus produced upward, rotatory and some horizontal eye movements but no downward eye movements. The present study has demonstrated

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MACULAE

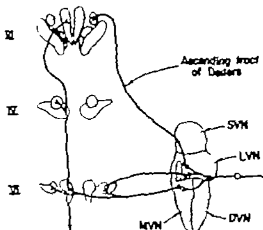


Fig. 59 Diagram summarizing the ascending pathways that may be activated by first order neurons from the maculae of utricle or saccule.

itory while the other is facilitatory allowing for a unidirectional eye movement.

In the second diagram (Fig. 59) a summary of the fibers from the otolithic sense organs, (utricle and saccule) could be represented by a fiber which has an ascending branch terminating in the ventral division of the lateral vestibular nucleus and a descending one terminating in the rostral portion of the medial vestibular nucleus. Compensatory eye movements whether they be horizontal or vertical from utricular stimulation or the theoretical vertical movements from saccular stimulation could be carried out by the systems in this diagram. For example, stimulation of the lateral rectus and medial rectus muscles via the direct connections of the medial and lateral vestibular nuclei to the abducens (VI) and to the medial rectus (III) via the ascending contralateral system is evident. Movements of the inferior rectus by way of the ascending tract of Deiters and the superior rectus by way of the contralateral MLF system from the medial vestibular nucleus could be the pathway for vertical eye movements from stimulation of the saccule or vertical portion of the utricle.

C. DIRECT CEREBELLAR CONNECTIONS TO THE EYE NUCLEI

The interesting and unique direct cerebellar projection to the trochlear (IV) nucleus and oculomotor (III) complex that was demonstrated in the control group confirmed the previous finding of Carpenter & Strominger (1964) and others (Allen, 1924 Van Gehuchten 1905 Gerebtzoff 1936, 1941 Klimoff 1898, 1899 Rasmussen, 1932). A clear demonstration of fibers of cerebellar origin coursing through the brachium conjunctivum was found terminating in the contralateral cells of the medial cell column of the oculomotor (III) complex and in a small portion of the contralateral trochlear (IV) nucleus. These innervate the ipsilateral superior rectus and superior oblique muscles respectively. Fibers reached these nuclear groups by passing in a dorsal direction from the region of the contralateral Red Nucleus and filtering at right angles through the longitudinally running fibers of the MLF.

The functional significance of this fiber connection is not clear but, as it is a unique connection, it is interesting to speculate on its significance. For example, the tremendous number of cerebellar fibers terminating in the superior rectus muscle group may be responsible for strong inhibition of this group and perhaps making movements in this direction less apparent upon peripheral stimulation, such as from stimulation of the saccule.

D. PROJECTIONS TO RETICULAR FORMATION

The various lesions in this group of animals consistently produced significant degeneration to the ipsilateral reticular formation. In most cases precise terminal and preterminal degeneration patterns around the larger neurons could be found (Fig. 60). This connection points up the well-known relationship between the vestibular system and the reticular

that the SVN projected to all the muscles supplied by nuclei III and IV except for the inferior rectus. This explains the absence of downward eye movements from stimulation in this nucleus.

Placement of the stimulating electrode in the middle portions of the vestibular complex which included the lateral vestibular nucleus and the rostral pole of the medial vestibular nucelus, produced upward rotatory horizontal and downward rotatory eye movements. Here the resulting eye reflexes were probably carried over the contralateral MLF ascending system to III and IV for upward rotatory movements, the projections to the abducens (VI) nuclei for horizontal movements, and over the ascending tract of Deiters to the inferior rectus group of III for downward eye movements.

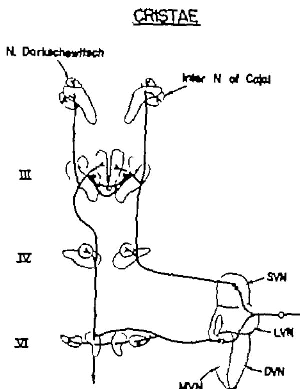


Fig 58 Diagram summarizing the ascending pathways that may be activated by first order neuron from the semicircular canal cristae.

B LABYRINTHINE-OCULAR PATHWAYS

Utilizing the data of previous studies which determined the termination in the vestibular nuclei of *primary* neurons from both the cristae and the maculae, two (2) summary diagrams correlating these ascending ocular systems with peripheral sense organs can be constructed.

In Fig. 58 the primary neuron from a semicircular canal crista is represented as a bifurcating nerve fiber sending an ascending branch to the superior vestibular nucleus and a descending one which terminates mainly in the rostral portion of the medial vestibular nucleus. These two nuclei connect the semicircular canal neurons to the III IV and VI nerve nuclei by way of the ipsilateral and contralateral ascending systems in the medial longitudinal fasciculi

The similarity in size and termination of the two ascending projections from the incoming first order neurons from cristae would appear difficult to understand in terms of eye movements in a particular direction. However there are several features of these pathways that would allow for well-coordinated directional eye movements from semi-circular canal stimulation Of course the distribution of fibers in each ascending system to nuclei supplying several eye muscles would indicate that probably several eye muscles are brought into action to produce even a relatively simple eye movement.

The length of the two ascending MLF systems is important in consideration of the physiology of this reflex. Obviously the contralateral ascending system from the descending branch of the primary vestibular neuron has a significantly longer path than the ipsilateral ascending system. This alone could produce a significant delay in the neuronal impulse traveling over this pathway as compared to the same impulse carried ipsilaterally to the III and IV nuclei This delay could prevent antagonistic action and allow for a directional eye movement.

Finally there is also the possibility that one of these two ascending pathways may be inhib-

MACULAE

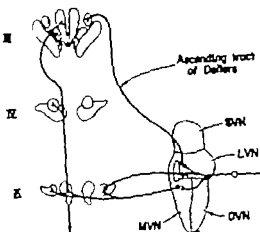


Fig. 59 Diagram summarizing the ascending pathways that may be activated by first order neurons from the maculae of utricle or saccule.

itory while the other is facilitatory allowing for a unidirectional eye movement.

In the second diagram (Fig. 59) a summary of the fibers from the otolithic sense organs, (utricle and saccule) could be represented by a fiber which has an ascending branch terminating in the ventral division of the lateral vestibular nucleus and a descending one terminating in the rostral portion of the medial estibular nucleus. Compensatory eye movements whether they be horizontal or vertical from utricular stimulation or the theoretical vertical movements from saccular stimulation could be carried out by the systems in this diagram. For example, stimulation of the lateral rectus and medial rectus muscles via the direct connections of the medial and lateral vestibular nuclei to the abducens (VI) and to the medial rectus (III) via the ascending contralateral system is evident. Movements of the inferior rectus by way of the ascending tract of Deiters and the superior rectus by way of the contralateral MLF system from the medial vestibular nucleus could be the pathway for vertical eye movements from stimulation of the saccule or vertical portion of the utricle.

C. DIRECT CEREBELLAR CONNECTIONS TO THE EYE NUCLEI

The interesting and unique direct cerebellar projection to the trochlear (IV) nucleus and oculomotor (III) complex that was demonstrated in the control group confirmed the previous finding of Carpenter & Strominger (1964) and others (Allen, 1924 Van Gehuchten, 1905 Gerebtzoff 1936, 1941 Klimoff 1898, 1899 Rasmussen, 1932). A clear demonstration of fibers of cerebellar origin coursing through the brachium conjunctivum was found terminating in the contralateral cells of the medial cell column of the oculomotor (III) complex and in a small portion of the contralateral trochlear (IV) nucleus. These innervate the ipsilateral superior rectus and superior oblique muscles respectively. Fibers reached these nuclear groups by passing in a dorsal direction from the region of the contralateral Red Nucleus and filtering at right angles through the longitudinally running fibers of the MLF

The functional significance of this fiber connection is not clear but, as it is a unique connection it is interesting to speculate on its significance. For example the tremendous number of cerebellar fibers terminating in the superior rectus muscle group may be responsible for strong inhibition of this group and perhaps making movements in this direction less apparent upon peripheral stimulation, such as from stimulation of the saccule.

D. PROJECTIONS TO RETICULAR FORMATION

The various lesions in this group of animals consistently produced significant degeneration to the ipsilateral reticular formation. In most cases precise terminal and preterminal degeneration patterns around the larger neurons could be found (Fig. 60). This connection points up the well-known relationship between the vestibular system and the reticular

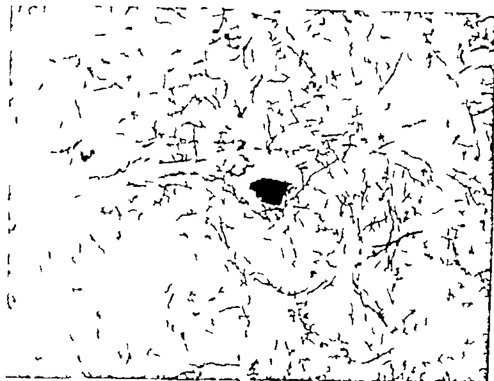


Fig 60 High power photomicrograph of a large neuron in the ipsilateral reticular formation in Cat 89 The degenerating terminals on the cell body and dendrites is typical of the lesions involving the vestibular nuclei in this study

formation and that patterned eye movements may be carried through the reticular formation.

However in this study as in any experiment where lesions are made in the vestibular nuclei interruption of cerebellar fibers (especially from the fastigial nucleus) as they pass through the vestibular nuclei to the reticular formation makes accurate interpretation difficult.

E. DESCENDING CONNECTIONS OF THE VESTIBULAR NUCLEI

Although this study concerned the ascending connections of the vestibular nuclei during the course of some lesions particularly in the caudal areas of the complex, descending fiber systems were interrupted. The diagram in Fig 61 summarizes the vestibular nuclei involved in the present experiment that also give rise to descending projections to spinal cord levels. General statements can be made at this time that the superior vestibular nucleus does not contribute to descending pathways to the cervical and lower cord regions. This contradicts the report by Buchanan (1937) in which it was stated that the superior vestibular nucleus does contribute to the lateral vestibulo-spinal tract. Lesions in the lateral vestibular nucleus consistently produced degenerating axons in the lateral vestibulo-spinal tract and only lesions in this nucleus produced degeneration in this tract this agreed with the studies of Brodal (1957 1962) and others (Buchanan 1937 Carpenter 1966) Lesions in the medial vestibular nucleus as indicated in the experimental animals, besides producing the ascending connections in the contralateral MLF also produced descending degenerating fibers in the medial portion of the contralateral MLF In many cases these apparently represent bifurcation of the fibers from the medial vestibular nucleus. Lesions in the descending vestibular nucleus were found to produce only descending connections by way of both the contralateral and ipsilateral descending MLF This group of fiber projections in a descending direction will be studied in more detail in another experiment.

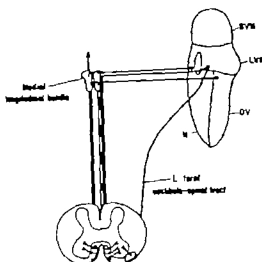


Fig. 61 Diagram summarizing the descending projections from the vestibular nuclei as determined from lesions in this study

F. VALUE OF SMALL NON-ELECTROLYTIC LESIONS

The importance of the smallest possible lesion in a complex area such as the vestibular nuclei for the evaluation of axonal degeneration was emphasized in this study. A non-electrolytic method using only a small straight probe produced such small lesions in the brainstem that the origin of the various fiber tracts could be determined.

The use of electric current usually produces relatively large lesions and always raises the question of current spread beyond the visible area of necrosis. Such large lesions make interpretation of degeneration patterns difficult and are probably responsible for conflicting results of other investigators in this area. Taking the superior vestibular nucleus as an example of how a large lesion can produce misleading degeneration patterns by injuring adjacent structures, the reports of Szentagothai (1964) and of Carpenter (1966) may be discussed. Knowledge of the direct cerebello-ocular fiber projection through the brachium conjunctivum to the superior rectus subgroup of the oculomotor (III) nucleus and to the contralateral trochlear (IV) nucleus is most important for proper evaluation of lesions in the superior vestibular nucleus. This was demonstrated in several animals in the present study. Interruption of this pathway by a large lesion of the SVN that also injures the adjacent brachium conjunctivum, is the most likely explanation for the findings of Szentagothai. He found degeneration primarily in the contralateral medial cell group (superior rectus) of III along with some degeneration to the ventral-lateral group of ipsilateral III (medial rectus) following a large lesion of the superior nucleus which unmistakably also involved the brachium conjunctivum. The conclusions drawn were that these represented the projection of the superior and horizontal semi-circular canals respectively.

In Carpenter's study degeneration in the oculomotor complex from superior vestibular nucleus lesions included the dorsal-lateral cell group which innervates the inferior rectus muscle. In the animals of the present study small lesions in all parts of the SVN conspicuously failed to produce degeneration in this part of the oculomotor complex. However, the ascending tract of Deiters, which is the only tract that terminates in this dorso-lateral cell group, does travel just ventral to the SVN as it ascends in the brainstem. The logical explanation for the discrepancy in results is that the relatively large electrolytic lesions in Carpenter's animals probably also injured the ascending tract of Deiters either directly or by spread of current beyond the confines of the superior vestibular nucleus.

G. NUCLEUS OF DARKSCHEWITSCH AND INTERSTITIAL NUCLEUS OF CAJAL

These two nuclei (Ingram, 1935) which lie just rostral to the oculomotor complex have been reported in several of the anatomical studies reviewed earlier to be the rostral termination of the ascending vestibular nuclear projections. This connection from both the ascend-

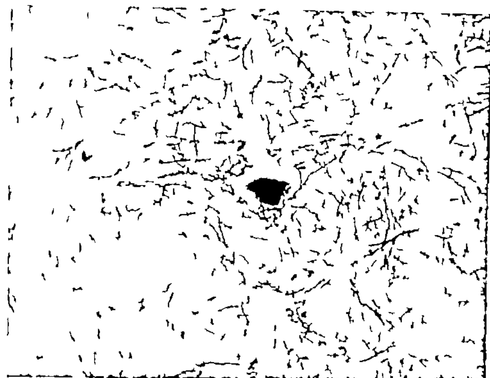


Fig 60 High power photomicrograph of a large neuron in the ipsilateral *reticular formation in Cat* 89 The degenerating terminals on the cell body and dendrites is typical of the lesions involving the vestibular nuclei *in this* study

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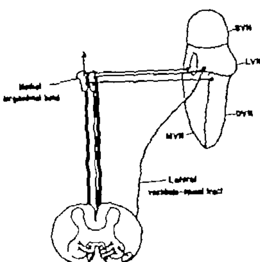


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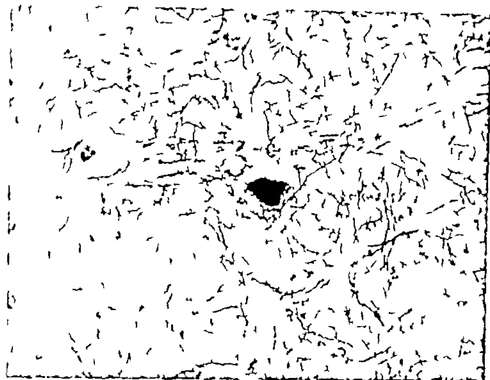


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Summary

Utilizing the Nauta silver method to impregnate axonal degeneration 6 to 7 days following small non-electrolytic lesions in the vestibular nuclei of the cat, ascending projections to the extra ocular nuclei were demonstrated. Reconstructions of the lesion and degeneration pattern to the III IV and VI nuclei in twenty-four cats were carried out:

Three ascending systems from the vestibular nucleiar complex were shown.

1 An ipsilateral projection which originates in the superior vestibular nucleus, travels in the lateral half of the MLF and terminates in the ipsilateral IV nucleus, bilaterally in all except the dorso-lateral group of the oculomotor complex, and finally in the nucleus of Darkschewitsch and interstitial nucleus of Cajal of the ipsilateral side. No connections with the VI nucleus are made from this system.

2. A contralateral projection which originates primarily in the medial vestibular nucleus, ascends in the medial half of the contralateral MLF and terminates in the IV III nuclei and the nuclei of Darkschewitsch and Cajal in a manner similar to the ipsilateral system. However connections to both abducens (VI) nuclei are formed by this pathway with the ipsilateral nucleus receiving a more prominent one than the contralateral. Some of the connections with the abducens nucleus are also given by the ventral portion of the lateral vestibular nucleus.

3. An ipsilateral system that travels lateral to the MLF and originates from the ventral portion of the lateral vestibular nucleus. This tract has been called the ascending tract of Deiters" and its terminus was shown to be the dorso-lateral cell group of the ipsilateral oculomotor complex. This area innervates the inferior rectus muscle.

Demonstrated also was the presence of direct cerebellar fibers coursing in the brachium conjunctivum and terminating in the contralateral trochlear (IV) and the medial cell group of oculomotor (III) nucleus. The eye muscles influenced directly by this system are, therefore, the ipsilateral superior oblique and superior rectus respectively

Descending projections in the MLF to the spinal cord from the medial, descending and lateral vestibular nuclei as well as the vestibulo-spinal projection from the lateral vestibular nucleus were also confirmed by lesions and degeneration patterns in these animals.

ing systems of the MLF was confirmed in the present study The further projections of these two nuclei are not known.

Brodal (1957 1960) on the basis of retrograde cell change in the interstitial nucleus of Cajal following transection of the MLF in the medulla, has suggested that at least some of the cells in the I C. projected in a descending direction through the MLF There was some indication that one terminus of these fibers was the medial vestibular nucleus. The interstitial nucleus of Cajal, therefore *may represent a feed back center to the vestibular nuclei.*

Virtually no information on the connections of the nucleus of Darkschewitsch is available. Information on the course and termination of these two nuclei must be gained from axonal degeneration studies following lesions in the midbrain involving these nuclei. Such lesions will be difficult to produce in this very inaccessible area into which other nuclei and fiber tracts are also crowded. These studies are planned in the near future.

It is possible that these two nuclei may represent the further projection of the central vestibular pathway to the cortical level. Despite electrophysiological demonstrations of a *cortical vestibular area (Anderson 1954 Mussopust* 1960 Walzl, 1949) anatomical evidence of this part of the pathway has never been presented

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SUPPLEMENT 294

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JOHN L. STEWART

From Santa Rita Technology Inc.,
Los Altos, California, USA

UPPSALA 1972

Introduction

Although basic to studies in psychoacoustics, there exists considerable misinformation on the mechanical properties of the cochlea, and data for use by investigators in devising instrumentation which behaves as does the ear are not readily available. Substantially different analyses for cochlear mechanics have too often only succeeded in perpetuating confusion as to the principles on which a model is based and just what a model can and can not do. This discussion is meant to provide clarification in four ways:

(1) A historical review of efforts at analyzing the cochlea is given in order to place the present work in proper perspective.

(2) Concepts of models for systems using electric circuits are discussed along with matters of relative accuracy and validity. It is shown how the final model results from a multi-step simplification of a complex three-dimensional lattice network. This section of the paper is partly tutorial.

(3) A specific numerical parameter set for an analog cochlea is provided which is suitable for extension and/or modification using conventional digital computer (time-share) techniques. The performance of the specific numerical model is shown with digitally computed characteristic patterns, impedances, and traveling waves. All curves given here are computed in this way. An appendix describes the computer program. An actual analog model has properties that are very close to these computed ones (within a few percent).

(4) The general nature of cochlear analysis is set forth using idealized functional representations. This part of the discussion is of basic importance in that it purports to deduce the basic rules for signal processing accomplished in the animal cochlea. This substantially nonspectral processing technique may also be accomplished in a multiplicity of neural structures central from the ear and also in nonauditory neural mechanisms. Cochlea-like analysis may have general applicability as an alternative to, or augmenter of Fourier methods.

A Short History

A number of analyses of the vibrations of the cochlea have been made in the past several decades and a few analog models have been constructed. Virtually all modern work in this area uses data deriving from the physiological studies of human preparations made by von Békésy (1960).

The earliest reasonably extensive effort to solve the problem of the cochlea dates from 1930 (where we ignore very early speculations based on a multiplicity of independent resonators). Theoretical formulations became more specific in the 1940's, with the work of Zwislocki (1948, 1949) and Ranke (1942, 1950) being singled out in particular. The works of Peterson & Bogert (1950), Bogert (1951), and

Elementary Functional Representations

When pictured as if rolled out into a straight line, the human cochlea appears as a tapered, fluid-filled tube which is separated into two channels with a structure having properties of mass and elasticity. This cochlear duct, containing the sensory cells, is bounded on one side with an elastic basilar membrane. The entire two-channel system is contained within presumably rigid walls. At the basal end it is closed except for two membrane-covered openings which provide access to the two channels. The stimulus is applied mechanically to one of these windows (the oval window). At the apex, the two channels inter-connect through a hole called the helicotrema.

In response to sinusoidal mechanical stimulation at the oval window fluids transmit pressure and particle velocity waves which move axially along the cochlear duct until a region is reached where duct mass and elasticity resonate so as to permit transverse motions between the two channels. The cochlea is said to localize the stimulus frequency in this region. The cochlea acts in the manner of a particular kind of hydrodynamic waveguide in which waves travel until reaching a cutoff region beyond which wave attenuation with distance is rapid.

The simplest concept of the cochlea assumes that fluids are constrained to move in only two directions, either axially along the cochlear duct or transversely to it. Equations of motion which countenance propagation as a sequence of events, increment by increment, can be formulated using this model. A general set of these is of second order for both fluid flow directions. That is, each has a first order differential, a first order integral, and a constant. When confined to linear systems, this assumption defines the (unbalanced) analog electric network structure of Fig. 1 (or its dual) wherein voltage and current represent pressure and fluid velocity respectively.

The network of Fig. 1 has been derived without considering any of the details of the cochlea itself. With use of one rather generalized boundary condition, an important modification results. Consider application of a constant pressure difference to the input with basal end windows being interpreted as simple holes. It is obvious from simple physical considerations that a steady fluid flow into one basal end window and out of the other will result. This means that none of the series string capacitors in Fig. 1 belong to the circuit representation if windows are ignored. But the membranous and elastic basal end windows prevent constant fluid flow in response to a pressure difference applied outside the windows. Representing this requires use of at least one series capacitor. Fig. 2 is for the modified network. This particular structure is the one generally used in representing the cochlea. The element values taper smoothly *from one end to* the other. An augmented basal end inductance accounts for comparatively large cochlear fluid volume (mass) near the windows, and the helicotrema resistance specifies hole size. The model does not allow for leaks between the two channels.

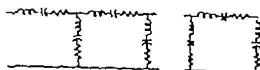


Fig. 1. General model for pair of second order difference equations representing law of physical activity

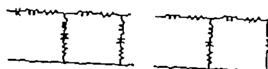


Fig. 2. General model allowing for continuous activity at arbitrarily low change rates except for one blocking element.

Fletcher (1951) appeared next Fletcher's early work (1930) is mentioned (but not in much detail) in his later book (1953)

In a 1953 historical survey Zwislocki (1953) observes that most theories differ not as to equations but rather as to particular parameter values, with those proposed by Fletcher (1951) being in best agreement with von Békésy's observations Certain analyses leading towards closed form solutions have necessitated use of approximations Zwislocki (1950) at one point ignores mass of the cochlear duct and Peterson & Bogert (1950) do not include dissipation Without damping a model will display wave *reflections* and act more like a cavity *than a* waveguide Fletcher (1951) points out the importance of dissipation in some detail

Studies subsequent to the foregoing which interpret system partial differential equations *of motion* for the cochlea provide little that is basically new These include our own earlier interpretation the main point of departure being that we used cochlear duct mass to infer elasticity *and* damping instead of relaying on von Békésy's hairprobe measurements We thus obtained a different set of parameters than found by other investigators (Caldwell et al 1962 Glaesser et al., 1963)

Several electric circuit analog models for the cochlea have been constructed based on data given in previously cited references The efforts of Bogert (1951) Bauch (1956) Wonsdronk (1961) and ourselves are cited In later versions of our own model, we abandoned the partial differential equation approach in favor of a topological one (Stewart 1967) This procedure does not constitute a denial of the validity of the differential equations rather it serves mainly to demonstrate the limitations of a simplified mathematical approach.

An analog ear structure of a different kind has been proposed by Flanagan (1960) He uses a delay line onto which are attached unsymmetric *bandpass filter* functions. He obtains *patterns* like those observed by von Békésy Flanagan's ear is a functional representation rather than a direct stimulation The individual bandpass filters do not separately distinguish effects *of outer and middle* parts of the ear from those due to the cochlea. The attenuation slopes of his filters above center frequency *may be too small*, although this particular restriction could be lifted with more complex bandpass functions. In general a *functional* model is not so capable of extension and *generalization* as is a direct stimulation (e g., in the representation of cochlear nonlinearities)

The problem of understanding cochlear mechanics has been approached by a *number* of investigators whose works are not so well known as those listed in the foregoing. For example Sakai & Ogushi (1968) provide a fairly detailed treatment. Studies are also given by Klatt (1964) Warnock (1964) Vallese (1964) and Klatt & Peterson (1966)

In addition to electric analog simulations, various attempts at building direct hydrodynamic models have been made commencing with razor blade glass-slide models described by von Békésy Such models are valuable for verifying and checking on physiological data, and can also aid in interpolation in cases where physiological data are difficult to acquire From a theoretical point of view however the scaled model is no more interpretable than is the cochlea itself and hence does not readily lead to a straightforward theoretical formulation for signal processing Even though the complete equations for the analog model may not be specified formally the very fact that the analog measures are quantitatively relatable provides a foundation of sufficient rigor for the needs of a satisfactory mathematical theory

Elementary Functional Representations

ben pictured as if rolled out into a straight ie, the human cochlea appears as a tapered, uid-filled tube which is separated into two hannels with a structure having properties of ass and elasticity. This cochlear duct, containing the sensory cells, is bounded on one ide with an elastic basilar membrane. The entire two-channel system is contained within presumably rigid walls. At the basal end it is closed except for two membrane-covered openings which provide access to the two channels. The stimulus is applied mechanically to one of these windows (the oval window). At the apex, the two channels inter-connect through a hole called the helicotrema.

In response to sinusoidal mechanical stimulation at the oval window fluids transmit pressure and particle velocity waves which move axially along the cochlear duct until a region is reached where duct mass and elasticity resonate so as to permit transverse motions between the two channels. The cochlea *is said to localize the stimulus frequency in this region.* The cochlea acts in the manner of a particular kind of hydrodynamic waveguide in which waves travel until reaching a cutoff region beyond which wave attenuation with distance is rapid.

The simplest concept of the cochlea assumes that fluids are constrained to move in only two directions, either axially along the cochlear duct or transversely to it. Equations of motion which countenance propagation as a *sequence of events*, increment by increment, can be *formulated using this model.* A general set of these is of second order for both fluid flow directions. That is, each has a first order differential, a first order integral, and a constant. When confined to *linear systems*, this assumption *defines the (unbalanced) analog electric network structure of Fig. 1 (or its dual) wherein voltage* and current represent *pressure and fluid velocity* respectively.

The network of Fig. 1 has been derived without considering any of the details of the cochlea itself. With use of one rather generalized boundary condition, an important modification results. Consider application of a constant pressure difference to the input with basal end windows being interpreted as simple holes. It is obvious from simple physical considerations that a steady fluid flow into one basal end window and out of the other will result. This means that none of the series string capacitors in Fig. 1 belong to the circuit representation if windows are ignored. But the membranous and elastic basal end *windows prevent constant fluid flow in response to a pressure difference applied* outside the *windows. Representing this* requires use of at least one series capacitor. Fig. 2 is for the modified network. This particular structure is the one generally used *in representing* the cochlea. The element values taper smoothly from one end to the other. An augmented basal end inductance accounts for comparatively large cochlear fluid volume (mass) near the windows, and the helicotrema resistance specifies hole size. The model does not allow for leaks between the two channels

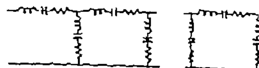


Fig. 1. General model for a pair of second order difference equations representing a line of physical activity.

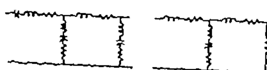


Fig. 2. General model allowing for continuous activity at arbitrarily low change rates except for one blocking element.

—this would require addition of certain elements such as resistor inductor circuits in parallel with the shunt resonant circuits

It is important to realize that the result has given almost no consideration to the details of the cochlea except for a rather broadly stated boundary condition The network can in fact represent a great variety of entirely different physical systems It is not really possible to closely identify network components in this model with parts of the cochlea For example, the shunt element resonant circuits may be only partly determined by the cochlear duct—for the influence of wall elasticity can also be represented here Series string elements may not directly represent channel fluid masses, but only a kind of equivalent mass, because particle velocities over the cross section of a channel may vary Response velocity may not be entirely represented by transverse current because this may also relate to wall rigidity and other factors and there will in any event be a location-dependent constant of proportionality relating this current to cochlear duct transverse response

Note on Approximation

The discrete lumped parameter network, or its ordinary differential equations which constitute an alternative description, comprises a more realistic model than does the set of partial differential equations for a continuous system The reason for this is simply that computations of a continuous system, whether by hand or with a computer must employ the difference equations which in fact relate to an equivalent lumped parameter system Furthermore even the development of the partial differential equation description is a limiting approximation of the discrete system The validity of the discrete representation for a system described with partial differential equations is in this sense beyond question But accuracy is another matter Accuracy depends upon the granularity of the increments, that is, the number of circuit elements used in the network Errors of representation depend upon the fractional difference between variables across a single increment or network section and this error can in principle be reduced without limit with a network of ever increasing complexity

A Note on Generalized Ladder Networks

The difference (or partial differential) equations leading to the structure of Fig. 1 comprise a rather general second order set. Thus the simple ladder network in Fig 1 has applicability to many modeling tasks However the ladder of Fig 1 is not a very general network Somewhat greater generality results upon replacing each separate resonant circuit with an arbitrarily complex impedance The only restriction on these impedance functions in order to guarantee physical realizability is that they be so-called positive-real functions.

A simple extension of Fig. 1 was briefly mentioned as a means for representing leaks through the cochlear duct But if the network shows elements representing these leaks or more general phenomena, then it could not come directly from the comparatively simple set of second-order difference equations. The description of continuous systems in science

seems most often to be in terms of fairly simple partial differential equations, and similarly for the corresponding difference equations. This would imply that many describable physical systems are either not really very complicated, or else partial differential equation representations as generally employed do not comprise very powerful tools. The procedure to be described next helps to demonstrate the power of semi-topological methods, a comparable derivation from the partial differential equations is not very tractable.

The Structural Model for a Rigid-Walled Pipe

An incompressable, nonturbulent fluid in a rigid-walled pipe behaves the same as a rigid mass insofar as overall input–output relationships are concerned. In particular for voltage and current the analogs of pressure and velocity respectively such a system can be represented as a single resistor in series with a single inductor. The inductor represents fluid mass and the resistor represents friction due to fluid viscosity the resistance value is zero for an ideal nonviscous fluid. If the incompressible fluid in the rigid-walled tube has input and output parts having different cross sectional areas, then the model additionally requires an ideal transformer to change the pressure to velocity (voltage to current) ratio

If flow over the cross section of a pipe is uniform and interest is in pressures and velocities at locations along the pipe, then a suitable model is a sequence of circuits in cascade each representing a small axial length. A pipe with changing cross section is represented as a sequence of simple resistance–inductance circuits interspersed with ideal transformers as in Fig. 3 The relationship of ideal transformer turns ratio to pipe cross section can be deduced from the principle of mass conservation The integral of fluid displacement, which is total fluid volume flow per unit time, must be constant.



Fig. 3 Representation for rigid-walled tube of varying cross section using ideal transformers.

Although useful for computational procedures, the model with ideal transformers is impractical in its literal analog form. A practical network requires removal of these transformers. In the case of circuits such as Fig. 3, this is possible using the device of impedance referral. That is, impedance elements can be moved from one side of an ideal transformer to the other as exemplified by the circuit in Fig. 4 A series of several ideal transformers can be removed by referring (somewhat tediously) across one section at a time until all such transformers are located at one end of the structure without intervening elements. Then all can be combined to a single ideal transformer The applied voltage is then simply scaled by the transformer and, since a simple scale factor is easily handled, even this one remaining transformer can be deleted. The final result is the simplified ladder in Fig. 5

If the fluid does not flow uniformly over the cross section of the pipe and if the state of fluids at points within the pipe is of interest, then a more detailed equivalent circuit must be employed. A small volume of incompressible fluid can be represented with partial differential equations and then subsequently modeled with a cubic array of resistor-inductor combinations. This representation has voltage and current as analogs for pressure and velocity respectively A large volume can be represented with many such circuits to give a space lattice where each lattice edge consists of a

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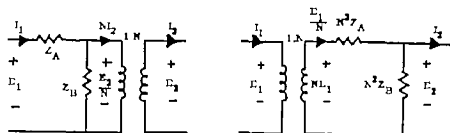


Fig 4 Referral of Impedances through an ideal transformer

series connection of one resistor and one in ductor A rigid boundary is represented as an open circuit for elements contiguous to this boundary A rigid walled tube of varying cross section is thus represented as a lattice of varying cross section and varying number of elements in each cross section. The elementary lattice element always represents a fixed volume A current injected at one end will flow with density inversely proportional to the number of elementary lattice units over the cross section and current magnitude in each series lattice element will similarly be inversely proportional to cross section Transverse elements contain currents only as needed to ac count for cross sectional changes To simplify this equivalent circuit to the form of a simple ladder is not quite so straightforward as in the case of uniform flow An equivalent aver age pressure and velocity over the cross sec tion of the pipe is implied by a simplified representation and shunt inductances are in troduced in order to represent transformer ac tion without transformers Each increment of length is thus represented as an el network of series R–L and shunt R–L. An ideal transformer with a 1 1 turns ratio can be identi fied with each series/shunt combination and combined in such a way as to change the turns ratio slightly and eliminate the shunt inductor This again results in a circuit as in Fig 3 which can subsequently be represented as in Fig 5 It follows that a rigid walled tube, uni form or tapered, carrying nonturbulent, in compressible fluids can be represented as a series of simple R–L combinations.

In order to be valid as a representation for increments along the tube the change in tube cross section per element of axial length must be small. This means that the step-up or step-down ratio of each transformer in Fig 3 must be small—for example 0 99 or 1 01 if errors are to be of the order of one percent It is also necessary that response variables represent only an overall average of fluid flow over the cross section of the tube because transverse currents are not accounted for in Fig 3

One of the restrictions on the foregoing model is that changes in cross section be gradual This implies that the pressure/velocity wavefront be substantially planar The re striction can be removed by separating the total fluid flow into stream tubes which individually have slowly varying cross sections. Each is treated as a separate rigid-walled struc ture It is helpful for present purposes to think of a rigid-walled tube starting and ending with

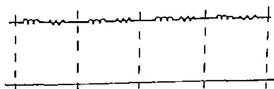


Fig 5 Representation for a rigid walled tube of varying cross section without use of ideal transformers. Element values taper according to variations in cross section. Position dependent scale factors must also be applied to voltage and current.

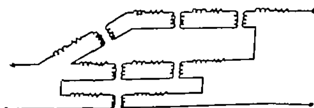


Fig 6 A hypothetical example of a representation for a three-channel system.

regions of slowly varying cross sections but which has intervening irregularities. Each streamtube will have the same number of sections (using the concept of conformal mapping) but numerical values for elements and transformer step-up or step-down ratios will vary. At the ends, all of the streamtube representations can be combined into a single one. Whether or not the system of ideal transformers can be eliminated by referral as before, the network remains a multi-path one as indicated in Fig. 6. Such a structure can not be described in simple terms with an elementary ladder network if details are to be retained. However if interest is confined to a sort of average system of pressures and velocities in the irregular region, the several pathways may be coalesced into a single one (providing the system does not involve separate distinct pathways).

Truly uniform fluid flow across a rigid-walled pipe is possible only for nonviscous liquids. This idealization does not constitute a good approximation except in some cases where cross sectional area is comparatively large. A streamtube approach is more appropriate for small pipes. But the final equivalent circuit can still imply an equivalent uniform flow and remain valid as to overall behavior.

The Problem of Nonrigid Walls

The resistance-inductance matrix representing a fluid region terminates in open circuits at rigid walls. For the case of non-rigid walls, terminations are impedance functions which can be simple or highly complex positive-real functions. With such terminations, transverse currents are important for structures with constant cross sections as well as for those with irregular ones. The separation of fluid flow into streamtubes is made extremely difficult because of the complex manner in which wall impedance functions can respond to pressure fluctuations. A yielding wall of any kind (if it is representative of a linear process) must always contain a measure of elasticity in its representation—which is capacitance in the circuit model. Necessity for capacitance is apparent if one considers what would happen otherwise: A constant pressure (voltage) to a resistor-inductor combination alone would result in constant velocity (current) flowing into the wall as long as the constant pressure was applied. It follows that, at very low frequencies, a wall will have the impedance of a capacitor and in the limit for a rigid wall the value for this capacitance goes to zero.

If a wall is very yielding, it has a circuit analog tending towards a short circuit—which contrasts with the open-circuit representation for a rigid wall. A tube with a yielding wall will not support flow that is uniform across the tube whether or not fluids are viscous. If the wall yields only when velocity fluctuations are within a restricted frequency range, then the wall capacitance must resonate with series-connected inductance; the equivalent mass of the wall provides the inductance parameter.

If a tube has a yielding wall on one side and a rigid wall on the other, the flow will tend to concentrate on the yielding side. If the tube has a large cross section, it may appear as if flowing waves are bound to the yielding part and remote fluids play almost no part in the process. The equivalent mass of moving fluids thus depends upon the nature of the boundary and in consort with wall elasticity the wave propagation velocity is also determined. (The equivalent of a yielding wall also describes behavior of so-called slow-wave propagation structures used in microwave circuits, and also certain kinds of electromagnetic and acoustic surface waves.) In hydrodynamic models of

the cochlea, von Békésy (1960) observed that cross section above a certain minimum value had little effect on motional patterns and activity was confined to a fluid layer adjacent to the elastic basilar membrane representation

A nonrigid wall becomes an array of resistor–inductor–capacitor circuits attached to points of the resistor–inductor space lattice representing fluids. The wall impedance elements can be highly complex and interconnected *within the wall itself* An especially complex situation arises when intrawall couplings are long range. A wave propagating along the tube at given velocity may be coupled via a re-entrant wall to establish itself ahead of or behind where it would occur if it traveled at normal velocity If this should prevail in a tube *which is intended to produce* space time patterns (like *the cochlea*) the formation of patterns would be inhibited An extreme example is given if walls of a closed tube (except for one end) move with the point of excitation—then nothing moves in the tube relative to its walls at all. (This is in part what occurs in the otosclerotic ear)

The Simplified Equivalent for the Cochlea

Two ducts are tapered, separated with a massive elastic structure and presumably have *rigid walls* Two suitably shaped space lattices *represent the two ducts. Walls are presumably* represented by open circuits but if these are not truly rigid, then more complex impedances for walls must be employed At least in approximation open circuits are assumed here

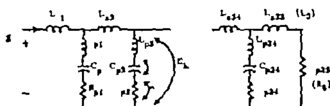
The cochlear duct is represented with an array of series resistor–inductor–capacitor circuits connecting appropriate points of the two space lattices together But since motion of a point on the cochlear duct will cause motion at adjacent points (because the duct is an integral structure) this array of duct connecting RLC circuits is in itself interconnected It is also complicated by variations in parameters between rigid edge attachments as well as by more gradual variations in the *axial direction*

To an approximation it would appear that intra-cochlear duct forces can be accounted for through suitable modifications of the edges of the fluid–representing resistor–inductor lattice which abuts the RLC cochlear duct representation Thus the structure appears the same as if no intra-cochlear duct forces exist but this is a fiction of equivalent circuits.

We now come to the task of reducing this rather complex three-dimensional circuit to a simple ladder as in Fig. 2 A gradual axial taper will require a series of ideal transformers. *Since the basilar membrane does not* have the same width as do the channels, a second set of transformers is needed to represent converging streamtubes in the transverse direction Clearly we must accept average velocities over the cross section and also an average one-dimensional RLC circuit array representation for the cochlear duct

After due manipulation, there results a balanced ladder network system having two sets of ideal transformers This structure can immediately be reduced to an unbalanced form Further (tedious) manipulations can work the two sets of transformers out of the system to give the network structure of Fig. 2 along with element values and necessary scale *factors relating voltages and currents* to fluid pressures and velocities, respectively

Inasmuch as there exist many uncertainties in the reduction process (as well as in parameters for the real cochlea) it is meaningless to retain very close numerical correspondence to such limited experimental data as are available. Rather a reasonable approximation that is not too difficult to express quantitatively is much to be preferred The approxima



b

sect.	L (mH)	L (Hy)	L (Hy)	C_p (F)	R_p (Ω)
1	403 3	3387	663988	8.33337E-04	76 4875
2	87	43706	13331	1.483 8E-03	334
	338 204	83036	7681 1	2.3448 E-03	84 481
	8 287	84114	8286	3.88836E-03	46 8
	1 1 930	78903	873967	6.63786E-03	33 8839
	89 383	88733	94364	8.85 3E-03	33 6827
	64.385	202334	1 64 97	1 32E-04	28.1839
	64 8325	17048	1 90843	2. 8030E-04	34 393
	40 1	332756	1 143	3.4344 E-04	20 7348
1	34.8343	247404	1 34802	3.300 E-04	17 6834
11	29 87	267873	1 33834	4.4778 E-04	3.831
12	23	287048	1 4353	33183 -03	13.7900
13	307	307820	1 53 1	2.07387E-03	8847
1	3. 33	370108	1 63084	3.33786E-03	0.38333
15	0 384	34804	1 77083	4.15800F-03	8821
1	867	70629	1 850	2. 303E-0	70744
1	30713	40 108	2 03384	1 27483E-02	70783
18	74343	30 77	2 13383	2.80348E-03	8571
1	68444	83 8	2 3400	7.1448 -03	333
20	8 83857	88796	2. 838	9438 E-02	3 1731
21	87 88	53841	2 3306	767353 -02	13
22	1 8348	77383	2 48479	13303	3 84700
23	1 848		875	71	8 48
2	1	844		38 20	84445
23		7			

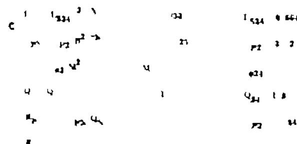


Fig. 7. Structure of a 24 section ear in ( ) also showing nomenclature for different response voltages. The resistors representing series coil losses are not shown. Table of normalized element values in (*b*). Parameter formulas in ( ).

tion is as follows. Except for terminating elements at basal and apical ends, all element values taper logarithmically from one end to the other. In particular, resonant frequencies of shunt circuits vary from 50 Hz at the apex to 20 000 Hz with Q values from 1.8 at the apex to 3.5. Inductance values in shunt circuits increase by a factor of 5 (logarithmically) from apical to basilar ends as do the series inductance values. Shunt inductors are larger than series inductors by a factor depending upon the total number of sections in the representation—the factor is 5 for 24 sections and 20 for 48 sections. Each series resistor inductor combination is such as to imply a Q value of 33.33 at 1 000 Hz. These several parameter value sets are regularized and smoothed versions of quantities estimated from physiological considerations (Stewart, 1967).

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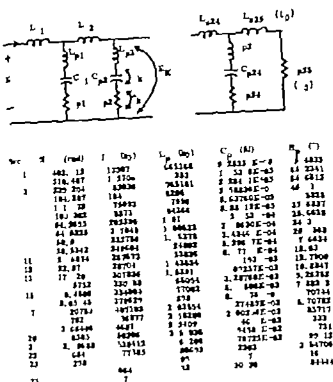


Fig. 7. Structure of 24 section ear in (*a*) also showing nomenclature for different response voltages. The resistors representing series coil losses are not shown. Table of normalized element values in (*b*). Parameter formulas in (*c*).

tion is as follows. Except for terminating elements at basal and apical ends, all element values taper logarithmically from one end to the other. In particular resonant frequencies of shunt circuits vary from 50 Hz at the apex to 20 000 Hz with Q values from 1.8 at the apex to 3.5. Inductance values in shunt circuits increase by a factor of 5 (logarithmically) from apical to basilar ends as do the series inductance values. Shunt inductors are larger than series inductors by a factor depending upon the total number of sections in the representation—the factor is 5 for 24 sections and 20 for 48 sections. Each series resistor-inductor combination is such as to imply a Q value of 33.33 at 1 000 Hz. These several parameter value sets are regularized and smoothed versions of quantities estimated from physiological considerations (Stewart, 1967).

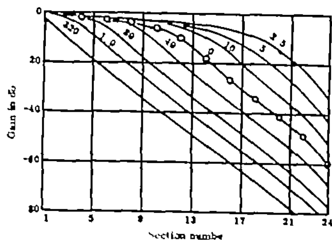


Fig 8 Series string voltage gain E/E_{in}. Parameter is normalized radian frequency Multiply by 50 for unnormalized frequency in Hertz. Circles are measured on a physical analog model (SRT model 407P).

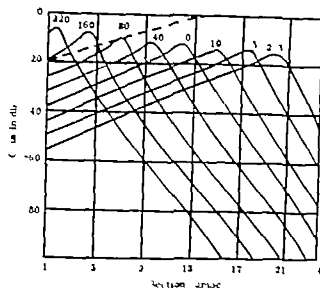


Fig 9 Responses across resistors representing transverse velocities. Parameter is normalized radian frequency

Computed Characteristics

An impedance and frequency normalized cochlea is useful for showing element values explicitly Fig 7 *a* shows the nomenclature for a 24 section cochlea (not including special basal elements) Fig 7 *b* is a table of element values, and Fig 7 c gives element value formulas.

The transverse velocity of the basilar membrane at a point along the basilar membrane is proportional to shunt circuit current, but *there exists a constant of proportionality that* depends upon index number K. Similarly transverse displacement is proportional to voltage across C_{pk} with an index-dependent constant of proportionality Initial studies of patterns can be made without considering these (frequency independent) constants.

A series of spatial patterns for various stimulus frequencies is shown in Figs. 8, 9 10, all relative to a normalized applied voltage of unity These (digitally computed) results are for total voltage across the shunt resonant circuits (cross-membrane pressure) total R_{pk} voltage (transverse velocity) and C_{pk} voltage (transverse displacement) The circles for the = 20 curve in Fig 8 are measured values for a physical analog network (SRT analog cochlear Model 2407P) Fig 11 is the input im

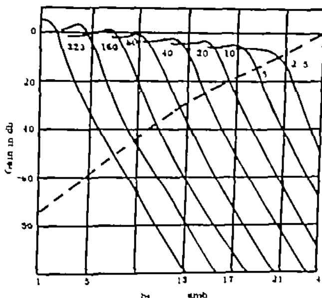


Fig 10 Responses across capacitors representing transverse displacement. Parameter is normalized radian frequency

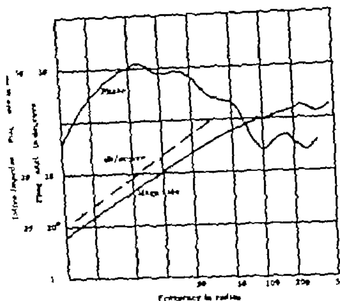


Fig. 11. Cochlea input impedance magnitude and phase.

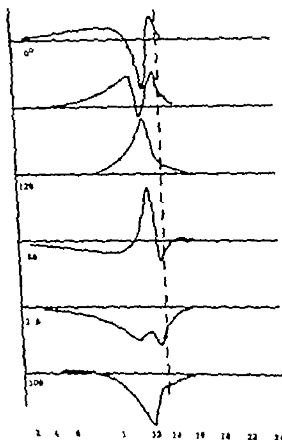


Fig. 12. Shape of 20 Hz traveling wave at 60° phase angle increments. The vertical dashed line shows the shunt circuit resonant frequency for 20 Hz.

pedance magnitude and phase for the entire structure, also digitally computed. An appendix describes the computer program used in making these calculations.

Computer solutions can be made to yield both magnitude and phase. This permits data to be obtained for plotting a traveling wave. Fig. 12 is the pattern for a 20 radian sine wave (1 000 Hz *unnormalized*) at six different relative phase angles. The location for 20 Hz resonance is also indicated. (Note: For a fixed sine wave frequency displacement and velocity traveling wave patterns have identical shapes because one relates to the other as a constant, $j\omega$.)

Frequency characteristics for total R_{pk} and C_{pk} voltages are shown at several different locations in Figs. 13 and 14. These do not include index number constants of proportionality nor do they include frequency effects of outer and middle parts of the ear. These latter effects increase rates of attenuation for higher frequency parts of these curves (above 80 Hz, which corresponds to 4 kHz), and decrease cut-off rates at lower frequencies (below about 16 Hz, which corresponds to 800 Hz).

Estimated index-dependent constants of proportionality which apply to total R_{pk} and C_{pk}

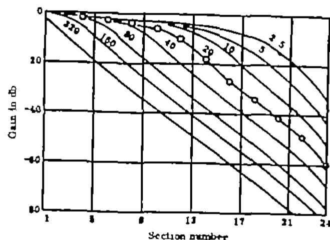


Fig 8 Series string voltage gain E/E_{in}. Parameter is normalized radian frequency Multiply by 50 for unnormalized frequency in Hertz. Circles are measured on a physical analog model (SRT model 2407P).

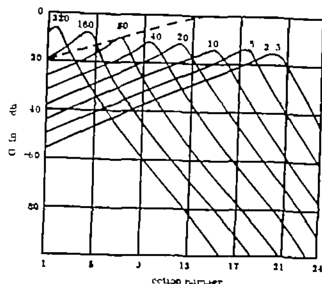


Fig 9 Responses across resistors representing transverse velocities. Parameter is normalized radian frequency

Computed Characteristics

An impedance and frequency normalized cochlea is useful for showing element values explicitly Fig 7 *a* shows the nomenclature for a 24 section cochlea (not including special basal elements) Fig. 7 *b* is a table of element values, and Fig 7 *c* gives element value formulas

The transverse velocity of the basilar membrane at a point along the basilar membrane is proportional to shunt circuit current but there exists a constant of proportionality that depends upon index number k. Similarly transverse displacement is proportional to voltage across C_{pk} with an index-dependent constant of proportionality Initial studies of patterns can be made without considering these (frequency independent) constants.

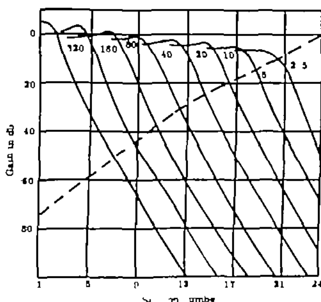


Fig 10 Responses across capacitors representing transverse displacement. Parameter is normalized radian frequency

A series of spatial patterns for various stimulus frequencies is shown in Figs. 8, 9 10 all relative to a normalized applied voltage of unity These (digitally computed) results are for total voltage across the shunt resonant circuits (cross-membrane pressure) total R_{pk} voltage (transverse velocity) and C_{pk} voltage (transverse displacement) The circles for the $\omega = 20$ curve in Fig 8 are measured values for a physical analog network (SRT analog cochlear Model 2407P) Fig 11 is the input im

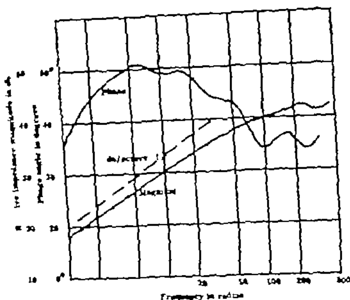


Fig. 11 Cochlea input impedance magnitude and phase.

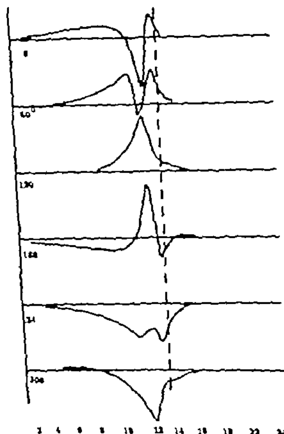


Fig. 12. Shape of 20 Hz traveling wave at 60° phase angle increments. The vertical dashed line shows the shunt circuit resonant frequency for 20 Hz.

pedance magnitude and phase for the entire structure, also digitally computed. An appendix describes the computer program used in making these calculations.

Computer solutions can be made to yield both magnitude and phase. This permits data to be obtained for plotting a traveling wave. Fig. 12 is the pattern for a 20 radian sine wave (1 000 Hz unnormalized) at six different relative phase angles. The location for 20 Hz resonance is also indicated. (Note. For a fixed sine wave frequency displacement and velocity traveling wave patterns have identical shapes because one relates to the other as a constant, $j\omega$.)

Frequency characteristics for total R_{pk} and C_{pk} voltages are shown at several different locations in Figs. 13 and 14. These do not include index number constants of proportionality nor do they include frequency effects of outer and middle parts of the ear. These latter effects increase rates of attenuation for higher frequency parts of these curves (above 80 Hz, which corresponds to 4 kHz), and decrease cut-off rates at lower frequencies (below about 16 Hz, which corresponds to 800 Hz).

Estimated index-dependent constants of proportionality which apply to total R_{pk} and C_{pk}

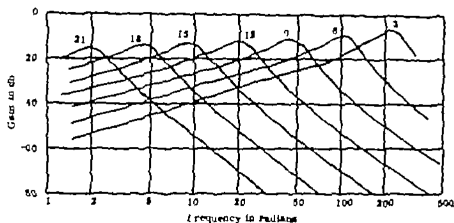


Fig. 13. Frequency characteristics across resistors. Parameter is section number.

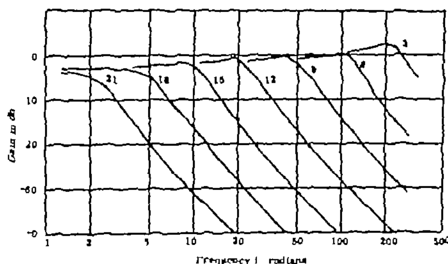


Fig. 14. Frequency response characteristics across capacitors. Parameter is section number.

voltages so as to yield transverse velocity and displacement, respectively are shown with dashed lines in Figs. 9 and 10. These curves are rather uncertain in the basal third of the cochlea. They have been estimated so as to yield the typical threshold characteristic (at cochlear pattern peaks) when a simplified representation for the transfer function of the outer and middle parts of the ear is employed.

Elasticity of the oval and round windows can be represented with a capacitor in series with L_{a} in Fig. 7 (7.5 E − 4 = 0.00075 fd). The attenuation across this capacitor is in the range 10–12 db per octave below about 16 Hz (800 Hz unnormalized). Except for transformer action this single element, as loaded by the cochlea, suffices to represent characteristics of outer and middle parts of the ear below about 40 Hz (2 000 Hz). Augmentation of L_{a} by a factor of several accounts for additional fluid masses near windows (from 0.133 to 0.39). With augmented L_{a} and the series capacitor a reasonable representation for outer and middle parts of the ear is given to frequencies at the upper end of the normal speech range. At higher frequencies, effects of ossicular mass and outer ear resonances cause further moderate changes to about 6 000 Hz, becoming increasingly marked as frequency increases beyond this.

General Discussion of the Model

The patterns provided by the specifically described analog model, and its input impedance, are in rather good agreement with von Békésy's (1960) observations. But patterns for frequencies above perhaps 40 Hz (2 KHz) are less certain. Recent measurements of a guinea pig cochlea suggest that Q values may become considerably larger at high frequencies (Johnstone et al., 1970). The present model allows for this to a certain extent by having Q values taper from 1.8 to 3.5. The generality of the equations permits an arbitrary taper rate and hence computer solutions could investigate what happens in this case. But with high Q values, a cochlea of only 24 sections may then not be adequate for representation near the basal end of the cochlea. Adequacy depends upon uses to which the analog model is to be put. If small differences between adjacent responses are of interest as, for example, in duplicating the tonal just-noticeable difference with these differences augmented by representations for neural inhibitory actions, then a very large number of sections is needed—enough to show discriminations of as little as 0.1 per cent in frequency. But if stimuli of interest are broadband signals such as speech up to perhaps 5 or 6 KHz (unnormalized), then the 24 section model should suffice. In terms of information content, it would appear that 24 sections is more than sufficient (Rink, 1970).

The analog model as discussed is a linear one. As such, turbulent fluid flow is not allowed; for a laminar flow model is needed in order to define streamtubes. It is believed by some that the cochlea vibrates with nonlinear motions to stimuli well within the normal range for speech perception. One concept is that of nonlinear fluid flow (Tonndorf 1960). The linear model then remains valid only if nonlinearities can be adequately represented with nonlinear components which are added to the linear system. Another concept has nonlinearities produced by asymmetric reaction of hair cells (Crane, 1966). This can be modeled with resistor-diode circuits placed across the C_{μ} elements so as to represent membrane displacement which is resisted more in one direction than in the other. Each such nonlinearity results in some envelope demodulation of lower frequency waves with these induced components then able to propagate along the cochlea, perhaps becoming augmented with contributions from other nonlinear circuits, as if the envelope component had existed in the applied stimulus. This type of nonlinearity is not of a simple "cubic" type. Rather the magnitude of the induced component is directly proportional to the magnitude of the stimulus.

This distributed nonlinearity representation may also provide a reasonable model for nonlinear fluid flow if this should in fact exist. The exact nature of the nonlinearities is actually not specified by the circuit representation.

A third concept for cochlear nonlinearity is that of asymmetric streaming of fluids in and around the oval window perhaps into the vestibular apparatus (von Békésy 1960). This concept can be modeled with a resistor-diode combination placed *after* the series *capacitor* representation for the oval window. It gives properties similar to those given with nonlinearities across the C_{μ} elements, but somewhat simpler in its effects on waveforms. The single diode-resistor combination has been used as a part of a model for the echoranging bat with some success (Stewart & Katson, 1969 Stewart, 1970). The distributed nonlinearity has been used in an attempt to explain *subjective* tones in human perception, also with some success (Stewart, 1970).

Cochlear nonlinearities could also be associated with nonrigid walls. To locate diodes with C_{μ} elements is thus somewhat mislead-

ing but not necessarily incorrect for in the simplified ladder network both the wall and the cochlear duct are accounted for with the same shunt circuit representation

Stimulation of the cochlea with bone conducted signals requires some re interpretation but not a change in the model itself At least for the linear model, the principles of reciprocity and superposition prevail In bone conduction elements of fluid throughout the cochlea are excited. But each elementary excitation can be referred to the input end by virtue of reciprocity then by virtue of the principle of superposition all of these incremental effects can be combined into a single equivalent stimulus at the oval window The difference between normal and bone conduction stimulation of a linear ear is thus explainable in terms of differences in the transfer functions between the source of sound and pressure on the oval window If the cochlea is nonlinear then neither reciprocity nor superposition apply except as approximations and to this extent bone conduction stimulation may not have an outer-ear equivalent.

The linear ladder network with general positive real functions for series and shunt arms, and whether balance or unbalanced is a so-called minimum phase network This means that given the magnitude transfer function from one series or shunt branch current or voltage to another then the imaginary part or real part or phase angle can be computed Or one can start with the phase function or the real part etc and compute all of the others. One technique for making trial computations manipulates the poles and zeros. Another employs the Hilbert transform (Bode, 1945) Applied to the present situation the minimum phase condition means that gain and phase and group delay (slope of the phase function) are entirely related. Thus a time-based interpretation is equivalent to a frequency based one and, unless concern is for waveforms more complex than sine waves, arguments supporting one interpretation over another are rather meaningless.

The rigid walled tube would appear to be representable with minimum-phase functions, even in its space lattice embodiment. A tube with yielding walls may or may not be so representable depending upon possible existence of reentrant pathways. Any structure having multiple paths between input and output such as a space lattice or paralleled ladder networks, may or may not be minimum-phase but a ladder network whose arms are positive real impedances is always minimum-phase. A distinctive nonminimum-phase structure could be one consisting of a yielding walled tube with a second one entering and leaving from different points in which wave travel times are significantly different over the two paths.

In a nonlinear structure most concepts relating to linear circuit analysis begin to break down This includes the concept of the minimum-phase property except of course, in approximation If nonlinearities create components but do not greatly change those already present or change induced components once these are in existence then the concept of minimum phase remains reasonably valid

Low Pass Filter Cascades

A study of the behavior of the cochlea with various idealizations leads to a fairly simple concept for method of analysis. That is, points along the basilar membrane respond to stimuli at the input to the system as a series of low pass filters whose cut-off frequencies are related by equal ratios (i.e., uniformly spaced on a log frequency scale) Fig 15 demonstrates these idealized transfer functions for a sequence of cochlear positions.

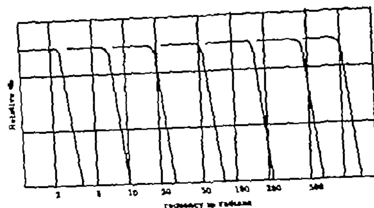


Fig. 15. A sequence of low pass functions as model for cochlear analysis. In this case, using the same normalization as previously capability beyond the normal human range is indicated by the use of filter function with cutoff frequency above 400 radius.

Response levels of the several low pass functions may not be the same. Amplitude weighting can be of two basic kinds, frequency-dependent and frequency-independent. A magnitude scale factor applied separately at each response location constitutes frequency-independent scaling. A common filter function which affects all response locations alike will vary in magnitude and phase with frequency and as such is frequency dependent. If the system of low pass functions is dependently scaled with a common multiplier $j\omega$ then an alternative point of view is that response becomes the time rate of change of what it was without the $j\omega$ factor. If the low pass function represents basilar membrane displacement, for example, then the representation with the $j\omega$ multiplier is of velocity.

A particularly interesting special case has the $j\omega$ factor and also an independent scale factor such that all peak responses are the same to give the filter system curves as in Fig. 16. If these curves are multiplied with a frequency-dependent bandpass function as shown dashed in Fig. 16, the overall system models the human ear. The dashed overall function represents the transfer characteristic of outer and middle parts of the ear including the oval-round window interface.

Cochlear localization patterns can be computed and plotted directly from the system of curves of Fig. 16. The idealized low pass system of Fig. 15 will also reveal localization provided that an independent scale factor is applied such that magnitude decreases with cut-off frequency (by about 50 db over the 1–400 Hz range, which corresponds to the normal range of 50–20 000 Hz).

The cut-off characteristics of the several low pass filters in Fig. 15 are not arbitrary. Ideally all curves are identical except for translation corresponding to frequency scaling. This assures that the phase characteristic from point to-point along the cochlea will be uniform and regular such that a wave will travel with gradually decreasing velocity until it disappears into an attenuating region. Since the cochlea models as a minimum phase function, this phase characteristic can be computed from the magnitude function of the low pass filter or conversely.

The most often accepted view of the cochlea is that of a frequency resolver. Indeed it does this to a certain extent, but it does not do it according to a classical filter band concept. In spectrum analysis, the phase function is usually ignored and it may not be under much control in any event. The way in which neural volleys are induced from a traveling wave along the basilar membrane can not readily be represented or even understood in terms of a classical frequency band concept. Rather gradual transitions from one position to the next become dominant factors in interpretation.

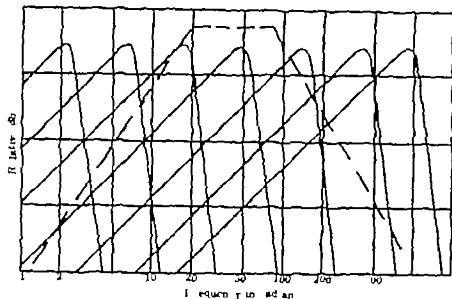


Fig 16 A sequence of overlapping bandpass functions as a model for cochlear analysis. *The multiplicative* outer-middle ear function is also shown (un-normalized corner frequencies being 1 000 Hz and 4 000 Hz).

Analysis of the cochlea in terms of low pass *bands is not a* generally accepted notion, al*though the traveling wave picture seems to be* becoming more common Innomata (1963) suggests *a low pass* band system. Peterson & Bogert (1950) and Fletcher (1951) might have discovered the low pass characterization from their pressure difference curves had they suitably accounted for complex arrangements of equivalent ideal transformers

Natural systems of many kinds support *traveling waves which may tend to disappear* after traveling a distance depending upon frequency Thermal and other diffusion processes behave in this way A diffusion of neural activity can also display this behavior and if this is combined with rate sensitivity a system of responses like those in Fig 16 but at a lower frequency could readily be mechanized In short, analysis of volleys of neural impulses in the central region can *take place* in analogy to analysis of sound volleys in the cochlea. Even a simple animal such as a small insect can have this capability The significance of cochlea like analysis has been recognized by a few investigators but much remains to be done (Lucas et al. 1966 Stewart, 1970 1971)

Summary

An electric circuit analog for basilar membrane vibrations is developed as a gradual *reduction of a complex three dimensional* hydrodynamic structure Specific circuits with element values are presented along with digital computer programs and numerical solutions. Information is sufficient to permit the reasonably competent designer/technician to construct his own circuit model Flexibility in design and generality of the theory allow for study of the effects of parameter changes by digital computer means The theoretical approach is meant to provide insight into both capabilities and limitations of the model and permit its extension and elaboration so as to represent additional phenomena The basic nature of the functional processing carried out by the cochlea is identified and described in terms of idealized response characteristics.

Appendix

DIGITAL COMPUTER PROGRAM

Element labeling is somewhat different from that previously described due to limitations in computer program designations. Analysis is of a rather general cascade of "L" network ladder sections. Fig. A 1 shows the general section where impedances are expressed in terms of real and imaginary parts, and Fig. A 2 shows the specific case of interest here. The individual L sections combine to form the complete network shown in Fig. A 3 (where differences in element nomenclature are to be noted).

The computer analyses begins by assuming a voltage across R0. The corresponding voltage and current relationships at the next section towards the input are computed and this procedure continues, section by section, until the input voltage and current are found. The magnitude of the input voltage is then used as a normalizing factor. The entire computation is repeated starting with the voltage across R0 which will result in an input voltage magnitude of unity.

The computer program is written in "Basic" language and the facility was a Hewlett Packard 2000A Time Share system. A telephone coupled terminal with a teletypewriter provided the input and output.

The program is shown in Fig. A 4 and the printout for the particular (normalized) frequency of 20 radius is shown in Fig. A 5. The computation of input impedance uses a simple self-evident modification of the program in Fig. A 4. To obtain data for the traveling wave, a different modification was done (not

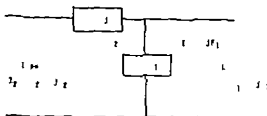


Fig. A 1. General equations for the "L" network.

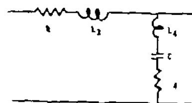


Fig. A 2. Specific ladder equations.

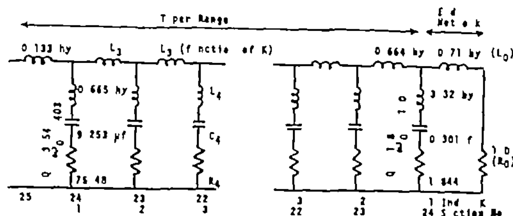


Fig A 3 Tapered analog car Not shown are loss resistors in series with L_4 elements. Loss in L_4 is accounted for by part of the one ohm of R_4.

```
10  PRI T
20  P INT "W ?
30  INPUT
40  PRINT
50  PRI T  SEC    "VO" "LINE DB  "R4 DB  "C4 DB
60  PRI T
70  Z 3
80  H 8 686
90  RO 1
100  LO  71
110  FO=1 E 15
120  FO 0
130  DATA  664, 9325 3 38  9325,1 00001 1 E78 1 8 1 03
140  FOR J 1 T  8
150  RI RO
160  XI   LO
170  EI FO-XI FO/RI
1 0  FI FO XI EO RI
190  UI RI 8 XI Q
200  FOR K 1 TO Z
210   ESTO E
220  READ L5 A,L6 B,W4,H 01 N
230  L3 L5 A (   1)
240  L4=L6 B  K 1)
250  WO       (  1)
260  Q Q1    (  1)
 70  C4=1/ L  W0 2)
 80   4=W0 L4/Q
290       L3
300      L
31    4=
320       L  1 (  C )
330  D        X4
340  E3  E1     2      4  1   D4
350  F    F1  4 2    R4  1   D4
 6   V3=H L  (  R F  C F3
  0  E5 (    F1   +C     4 F   V+C  ) D4
3 0  F5= (      I     C     R   I     C     D4
39    5= +LOG              F
400   I H L 0( Q  E1    F1
 10  IF J 1 TH   430
420    I T        V0     V3 V5
430  D=  4  I  2        X1)
440       (               I      4+                E)/
450       ( I  4 E                    )      1 R     /
460  D1 ( R-K               R
  0       E (R2 R        (       )
4     T          2 2)       X   X  / 1
  0  E  E1         T
500  F2 F   3 F   T
510      L
52    1 F
530
540   1   8
 50   EXT
560   O=           F       1 R)
     F0=E0 E1 (E1 R F1 R
      E T J
59     1
600    I T
 10    T
  0  END
```

Fig A 4 Computer program in Basic Language.

shown here) in which the relative phase angle of the stimulus was also a parameter

Program statements, in major groups, are described as follows.

20 30	Input frequency typed in
50	Printout table headings.
70	Number of sections.
80	Nepers to decibels.
90 100	End section values.
110–120	First assumption on voltage at R0 An overflow can be handled by changing the value of E0
130	Element taper constants (can be anywhere in program)
140	Start with normalizing loop.
150–190	End section computations.
200	Start section stepping
230–300	Taper equations.
310–400	Compute for one L section.
410	Skip printing on normalizing loop.
420	Print data (this can be expanded in many ways)
430–500	Compute at next section towards input
510–540	Relable for next section
550	Section number loop
560 570	Adjust output volts for $1 + j0$ input.
580	Normalizing loop
610	Return for a new frequency

End section values can be changed over wide limits in 90 and 100 in order to answer such

Fig. A 5. Numerical printout sample for $\omega = 20$.

questions as the effect of the equivalent impedance for the helicotrema. Data in statement 130 can be changed to study effects of taper rates and relevant constants (see Read statement 220 for definitions of constants). The Q of the series elements is set in statement 290.

Print statement 420 can be modified in many ways, as can table headings, etc. For example, curves of several variables, in decibels, can be printed directly by the terminal as functions of section number using different teletypewriter symbols for the different curves. But these things constitute conveniences which are not important here.

Even though a comparatively inexpensive time sharing service was employed, apparent speed of computations is maximal. That is, central processor delay is less than the time required to type table headings. A representative computer connection cost for the data in Fig. A 5 is in the range 15 to 60 cents, depending on time of day, facility and other factors (or even less using Image statements so as to get more compact printout).

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Johnstone, B. M., Taylor, K. J. & Boyle, A. J. 1970. Mechanics of the Guinea pig cochlea. *J. Acoust. Soc. Amer. 47* 504–509.

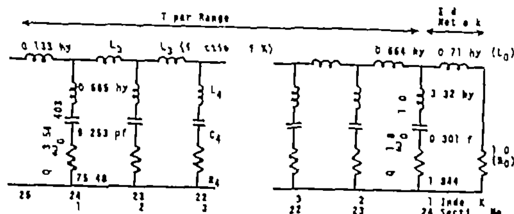


Fig A 3 Tapered analog ear Not shown are loss resistors in series with L_4 elements. Loss in L_4 is accounted for by part of the one ohm of R_4.

```
10  PRINT
20  PRINT "W ?"
30  INPUT W
40  PRINT
50  PRINT "SEC #" "WO" "LINE DU" "RA DU" "C4 DB
60  PRINT
70  Z 3
80  H 8 686
90  R0 1
100 L0 71
110 F0=1 E 15
120 F0 0
130 DATA   6   93-5 3 32   9325 1 00001 1 098 1 8 1 03
140 FOR J 1 TO 8
150 R1 0
160 X1 W*L0
170 E1 F0+X1 F0/ 1
180 F1 F0+X1 L0/R1
190 D1 1 E X1 Q
200 FOR K 1 TO Z
210 RESTORE
220 READ L5,A,L6 B   H 01 N
230 L3 L5 A (K 1)
240 L4=L  B  K 1)
250 W0        K 1)
260 Q=01    (   1)
270 C   1  L   0 C)
[illegible]
```

Fig 4 4 Computer program in Basic language.

shown here) in which the relative phase angle of the stimulus was also a parameter

Program statements, in major groups, are described as follows:

20 30	Input frequency typed in
50	Printout table headings.
70	Number of sections.
80	Nepers to decibels.
90 100	End section values.
110–120	First assumption on voltage at R0. An overflow can be handled by changing the value of E0
130	Element taper constants (can be anywhere in program)
140	*Start with normalizing loop*
150–190	End section computations.
200	Start section stepping
230–300	Taper equations.
310–400	Compute for one "L" section.
410	Skip printing on normalizing loop.
420	Print data (this can be expanded in many ways)
430–500	Compute at next section towards input
510–540	Relable for next section.
550	Section number loop
560 570	Adjust output volts for $1 + j0$ input.
580	Normalizing loop
610	Return for a new frequency

End section values can be changed over wide limits in 90 and 100 in order to answer such

Fig. A 5. Numerical printout sample for $\omega = 20$.

questions as the effect of the equivalent impedance for the helicotrema. Data in statement 130 can be changed to study effects of taper rates and relevant constants (see Read statement 220 for definitions of constants). The *Q* of the series elements is set in statement 290

Print statement 420 can be modified in many ways, as can table headings, etc. For example, curves of several variables, in decibels, can be printed directly by the terminal as functions of section number using different teletypwriter symbols for the different curves. But these things constitute conveniences which are not important here.

Even though a comparatively inexpensive time sharing service was employed, apparent speed of computations is maximal. That is, central processor delay is less than the time required to type table headings. A representative computer connection cost for the data in Fig A 5 is in the range 15 to 60 cents, depending on time of day facility and other factors (or even less using Image statements so as to get more compact printout).

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Introduction

The posterior colliculus is an imposing part of the auditory system in the midbrain tectum. It functions in the propagation of auditory signals to the medial geniculate body (Bechterew 1895), in the integration of the ascending and descending auditory pathways implicated in central auditory "feedback" adjustments (Rasmussen, 1964 Desmedt, 1960 Massopust & Ordy 1962), and in the elaboration of acoustic reflexes (Ades, 1944 Buser St. Laurent & Menini, 1966). All of the known auditory pathways undergo obligatory synaptic interruption at the level of the posterior colliculus (Lewandowsky 1904 Rasmussen, 1964 Morest, 1965 *b*). *It is important to comprehend the detailed structure of the posterior colliculus, since it subserves several different auditory functions* and since a *significant analysis of sound may already be achieved at this level* of the auditory system (Neff 1961). Moreover the tonotopic organization of the auditory nuclei (Rose, Galambos & Hughes, 1959 Rose, Greenwood, Goldberg & Hind, 1963 Tsuchitani & Boudreau, 1966 Guinan, 1968), which corresponds to the orderly arrangement of axons in these regions, including the medial geniculate body (Morest, 1965 *a*) and the posterior colliculus (Morest, 1964 *b*) of cats, implies an orderly arrangement of the neurons in the posterior colliculus of the human also.

Unfortunately the desired picture of the minute structure of the posterior colliculus is not provided by the available histological studies, which are often limited to the Nissl and Weigert methods (e.g., Winkler 1921 Riley 1943), with the provocative exception of a few precious observations with the Golgi method by Ramón y Cajal (1911) in the mouse, cat, and dog. Recently a detailed analysis of the neuronal architecture of the posterior colliculus has been pursued in the cat with the Golgi method and with experimental degeneration methods (Morest, 1964 *b* 1966 *a*, 1966 *b*). This work has demonstrated an orderly laminar arrangement of the axons and dendrites of the central nucleus of the posterior colliculus that may account for the tonotopic organization of the ascending auditory pathway in the midbrain. A cortical structure has also been characterized that could provide for the complex integration of the ascending and descending auditory pathways. Peri-collicular groups of neurons, some of which could integrate somatic sensory and auditory inputs, have also been identified.

The present investigation provides a detailed description of the neuronal architecture of the human posterior colliculus. This study should help to define the possible functional significance of the human posterior colliculus by facilitating references to the experimental analyses of the homologous structures in the cat.

Materials and Methods

The analysis was made on Golgi-Cox impregnations (Van der Loos, 1959) of fifteen human midbrains, ranging in age from 24 weeks of gestation to 74 years (see Table I). The fresh tissue was cut into slices 3–5 mm thick and immersed in the Golgi-Cox fixative,

List of Abbreviations

AC	Anterior colliculus	IT	Intercollicular tegmentum
AX	Commissure of anterior colliculus	LL	Lateral lemniscus + dorsal nucleus of lateral lemniscus
C	Cuneiform area	LZ	Lateral zone
CC	Caudal cortex	MLF	Medial longitudinal fasciculus
CG	Central gray	MT	Mesencephalic trigeminal tract
CN	Central nucleus	PC	Posterior colliculus
CX	Commissure	T	Trochlear nerve
DC	Dorsal cortex	Vln	Ventrolateral nucleus
DM	Dorsomedial nucleus	I–IV	Layers of cortex of posterior colliculus
IC	Intercollicular commissural zone		

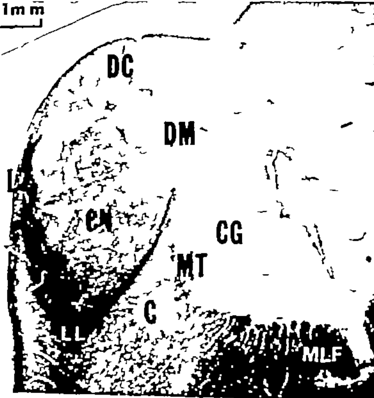


Fig. 1. Frontal section at the junction of the caudal and middle thirds of the posterior colliculus of an adult. Loyez method.

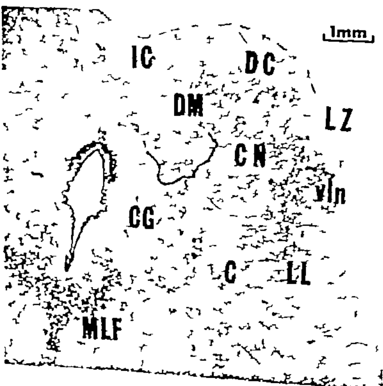


Fig. 2. Frontal section at the junction of the caudal and middle thirds of the posterior colliculus of an adult. Cresyl violet.

Table I

Age	Plane of section	Cause of death	Time of fixation after death (hours)
24 week fetus[a]	Horizontal	Spontaneous abortion	1
25 week fetus	Sagittal	Stillbirth	/
9 months	Sagittal	Tetralogy of Fallot	-
29 years	Frontal	Gram negative septicemia	3 /
46 years	Frontal	Myocardial infarction	-
51 years	Sagittal	Pneumonia	4
51 years	Horizontal	Chronic lung disease	4 /
52 years	Frontal	Nephrosclerosis	1 /
53 years[a, b]	Sagittal	Rectal carcinoma pneumonia	1 /
55 years[a]	Frontal	Congestive heart failure	2
62 years	Sagittal	Thoracic trauma	4
63 years	Sagittal	Pulmonary insufficiency	5
69 years	Frontal	Myocardial infarction	3
70 years	Horizontal	Pulmonary embolism	4
74 years	Frontal	Pneumonia	2

Used for serial reconstructions.
[b] *Counterstained with cresyl violet (Ramon-Moliner Vane & Fletcher 1964).*

usually less than four hours after death After celloidin embedding unbroken series of sections, 150 μ thick were made in one of the three standard planes frontal sagittal and horizontal The distribution of the neurons impregnated in each of these planes was reconstructed from serial sections on transparent plastic sheets with the aid of photomicrographs and a stereomicroscope with a drawing attachment. The drawings were made with a compound microscope having semi-apochromatic objectives and a drawing attachment. The observations were compared with preparations stained by the cresyl violet Klüver and Loyez methods,[1] and with Golgi-Cox impregnations of adult cat brains sectioned in the three standard planes. This report adheres to the conventions of comparative anatomy The rostral and caudal directions are designated as anterior and posterior respectively the back and front as dorsal and ventral. The findings exemplify the posterior colliculus in the mature brain unless otherwise indicated

Results

The three main divisions of the posterior colliculus are readily apparent in the Golgi preparations, as well as in the myelin and cell stained material the central nucleus, the cortex, and the peri-collicular tegmentum (Figs 1–7) The central nucleus (*CN*) is an ovoid mass of cells, occupying the core of the posterior colliculus The nucleus is separated from the surface of the collicular convexity by the cortex dorsally (*DC*) and caudally (*CC*) The peri-collicular tegmentum (*IT LZ C* respectively) separates the central nucleus from the anterior colliculus, from the lateral surface of the tectum and from the underlying regions of the midbrain tegmentum Most of the heavily myelinated tracts, running to and from the central nucleus, collect in the peri-collicular teg

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[1] This material was generously made available by Dr Bruce Konigsmark, The Johns Hopkins University

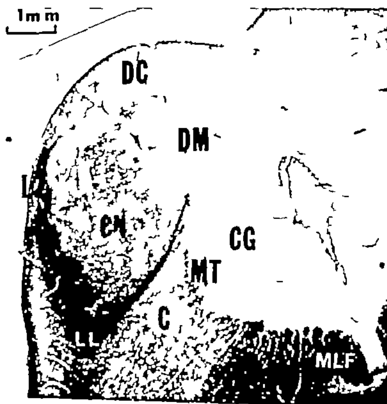


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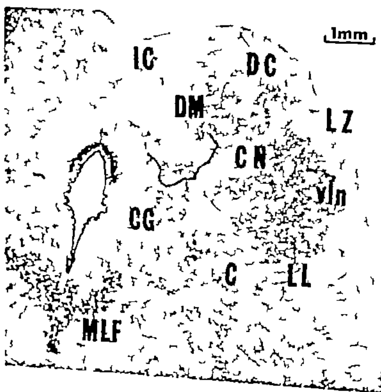


Fig. 2. Frontal section at the junction of the caudal and middle thirds of the posterior colliculus of an adult. Cresyl violet.

Table 1

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24 week fetus[a]	Horizontal	*Spontaneous abortion*	1
25 week fetus	Sagittal	Stillbirth	1
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29 years	Frontal	*Gram-negative septicemia*	3 ¼
46 years	*Frontal*	Myocardial infarction	2
51 years	Sagittal	Pneumonia	4
51 years	Horizontal	Chronic lung *disease*	4 /
52 years	*Frontal*	Nephrosclerosis	1 /
53 years[a, b]	*Sagittal*	Rectal carcinoma pneumonia	1 /
55 years[a]	*Frontal*	*Congestive heart failure*	2
62 years	Sagittal	Thoracic trauma	4
63 years	Sagittal	Pulmonary insufficiency	5
69 years	Frontal	Myocardial infarction	3
70 years	Horizontal	Pulmonary embolism	4
74 years	*Frontal*	*Pneumonia*	2

[a] Used for serial reconstructions.
[b] Counterstained with cresyl violet (Ramon-Moliner Vane & Fletcher 1964).

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Fig. 5. Parasagittal section at the junction of the medial and middle thirds of the posterior colliculus of a 53 year old man. The rostral direction is toward the top of the field, the dorsal direction is at the right. Note rostro-caudal striae in central nucleus corresponding to dendritic laminae. Golgi-Cox.



Fig. 6. Parasagittal section at the junction of the lateral and middle thirds of the posterior colliculus from the same brain as the preceding figure. Note rostro-caudal striae in central nucleus corresponding to dendritic laminae. Golgi-Cox.

mentum and in the superficial cortex, so that in myelin-stained sections the central nucleus seems to be encapsulated (Fig. 1). The brachium of the posterior colliculus, containing the axons ascending to the medial geniculate body and descending from the auditory cortex, appears in the lateral zone (*LZ*) of the colliculus, whereas the lateral lemniscus (*LL*), containing the ascending auditory axons to the central nucleus, gathers at the posteroventral pole of the *central nucleus*. The *commissure* of the posterior colliculus (*CX IC*) contains myelinated axons passing between the posterior colliculi over the cerebral aqueduct and central gray (*CG*) at the level of the trochlear nucleus.

The central nucleus consists of a mass of densely packed nerve cell bodies of many sizes and shapes (Figs. 7, 8, 11), intermingled as if at random. We have not attempted to delineate subdivisions of the central nucleus, as in the cat (Morest, 1966 *b*). However, in Golgi impregnations it is possible to distinguish several types of neurons on the basis of their dendritic morphology. These types are not intermingled haphazardly, but they are arranged in a specific order. This order is defined by the spatial orientation of the dendrites of the different types of neurons. There are at least five types of neurons, two of which have disc-shaped *dendritic fields* (*Figs. 8, 9, 10*) and three of which have stellate dendrites (Figs. 16, 17, 18).

The disc-shaped neurons line up with their

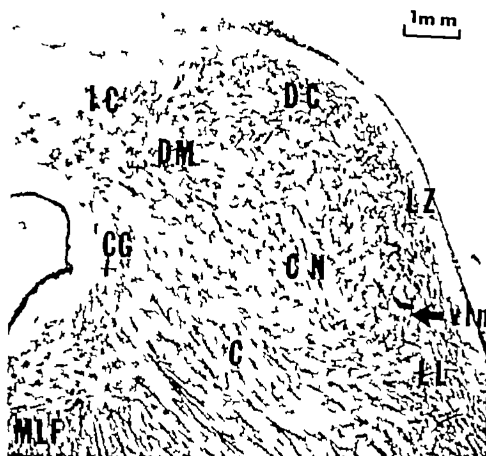


Fig 3 Frontal section at the junction of the caudal and middle thirds of the posterior colliculus of a 55 year old man Note lack of impregnation of the superficial zone or capsule of the colliculus, as is characteristic of the present series. Golgi-Cox method.

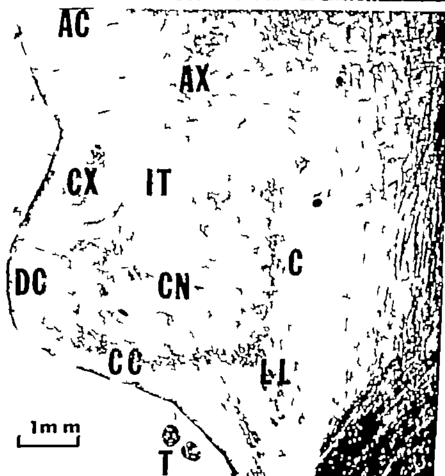


Fig 4 Parasagittal section at the junction of the medial and middle thirds of the posterior colliculus of an adult. The rostral direction is toward the top of the field, the dorsal direction is at the left. Luxol fast blue cresyl violet method.

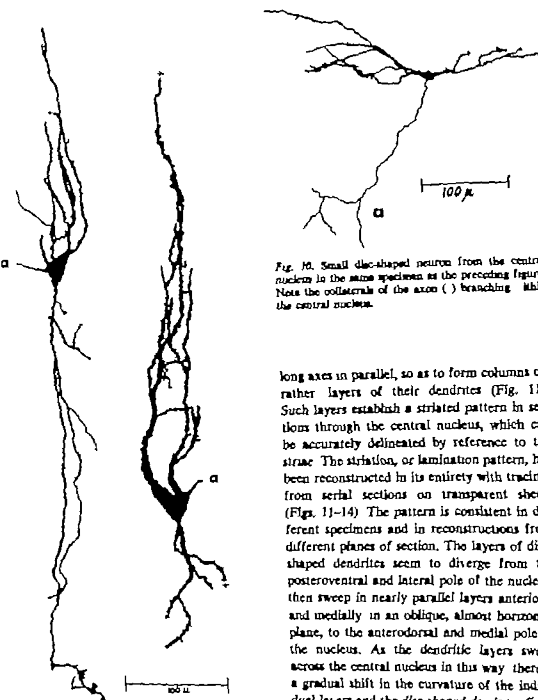


Fig. 10. Small disc-shaped neuron from the central nucleus in the same specimen as the preceding figure. Note the collaterals of the axon ( ) branching within the central nucleus.

Fig. 9. Two large disc-shaped neurons from the central nucleus of a 53 year old man. Note the dendritic growth cone on the tip of the dendrite of the left-hand cell (bottom edge of field). Parasagittal section. Golgi-Cox.

long axes in parallel, so as to form columns or rather layers of their dendrites (Fig. 11). Such layers establish a striated pattern in sections through the central nucleus, which can be accurately delineated by reference to the striae. The striation, or lamination pattern, has been reconstructed in its entirety with tracings from serial sections on transparent sheets (Figs. 11–14). The pattern is consistent in different specimens and in reconstructions from different planes of section. The layers of disc shaped dendrites seem to diverge from the posteroventral and lateral pole of the nucleus, then sweep in nearly parallel layers anteriorly and medially in an oblique, almost horizontal plane, to the anterodorsal and medial pole of the nucleus. As the dendritic layers sweep across the central nucleus in this way there is a gradual shift in the curvature of the individual layers and the disc-shaped dendritic fields forming them (Figs. 14, 15). The number of individual layers, or the number of neurons *forming a functional laminar unit,* is not *known,* since there is no obvious structural boundary between the layers. Indeed, the dendrites *of the individual* cells always overlap

IC

ventrad

DC

I II III IV

DM

IT

CN

LZ

C

1mm

vlp

LL

Fig 7 Drawing of cell groups constructed from two adjacent frontal sections at the junction of the caudal and middle thirds of posterior colliculus. One of the sections appears in Fig. 3 Dashed line indicates the boundary of the central nucleus. The boxed areas, beginning with DC, are shown at higher magnifications in Figs. 20 and 30–33



Fig 8 Photomicrograph of small (a) and large (b) disc shaped neurons of the central nucleus in an adult. Note highly polarized dendrites. Parasagittal section with the rostral direction at the right

Fig. 12. Photomicrograph of parasagittal section at the junction of the middle and lateral thirds of the posterior colliculus showing impregnation of the dendritic laminae of the central nucleus and the cellular layers of the cortex from the same specimen as in the preceding figure. *Inset*, orientation of the dendritic laminae in the central nucleus. The dashed lines demarcate the sector of the posterior colliculus show in the photomicrograph.

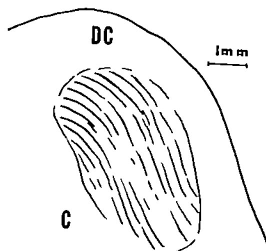


Fig. 13. Orientation of the dendritic laminae from the central nucleus, as seen in the frontal section show in Fig. 3.

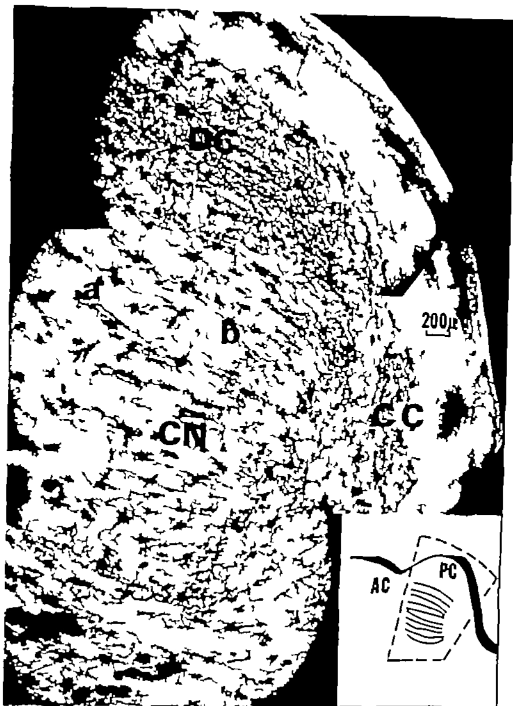


Fig 11 Photomicrograph of a parasagittal section at the junction of the medial and middle thirds of the posterior colliculus showing impregnation of the dendritic laminae of the central nucleus and the cellular layers of the cortex. *a* large disc-shaped neuron (also shown in Fig. 9 right) *b* small disc shaped neuron (shown also in Fig. 10) *Inset* orientation of the dendritic laminae in the central nucleus. The dashed lines demarcate the sector of the posterior colliculus shown in the photomicrograph Golgi-Cox 53 year old man

with those of adjacent cells both within and across the plane of the layer The dendritic layers are paralleled by the incoming fibers of the lateral lemniscus This lamination of the disc shaped dendrites is analogous to that previously described for the disc shaped neurons of the medial geniculate body (Morest, 1965 *a*) and posterior colliculus (Morest 1964 *b*) in the cat except that in the former case the pattern is more complicated and in

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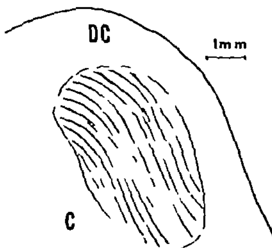


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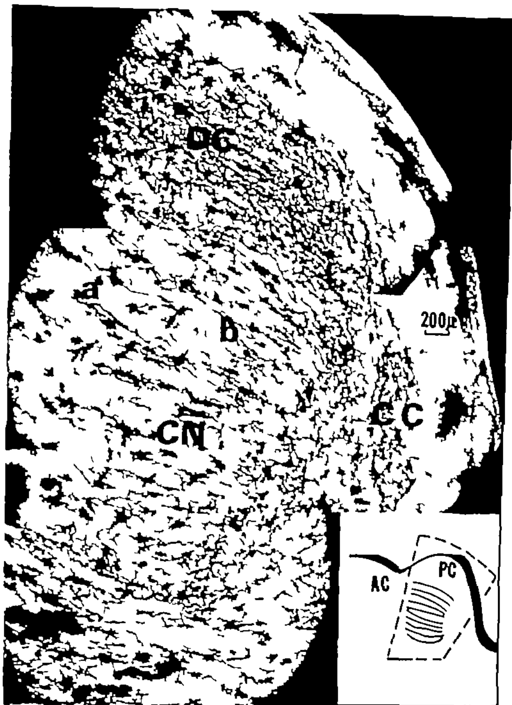


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with those of adjacent cells both within and across the plane of the layer The dendritic layers are paralleled by the incoming fibers of the lateral lemniscus This lamination of the disc shaped dendrites is analogous to that previously described for the disc shaped neurons of the medial geniculate body (Morest 1965 *a*) and posterior colliculus (Morest 1964 *b*) in the cat except that in the former case the pattern is more complicated and in

Fig. 12. Photomicrograph of paramagittal section at the junction of the middle and lateral thirds of the posterior colliculus showing impregnation of the dendritic laminae of the central nucleus and the cellular layers of the cortex from the same specimen as in the preceding figure. *Inset*: orientation of the dendritic laminae in the central nucleus. The dashed lines demarcate the sector of the posterior colliculus show in the photomicrograph.

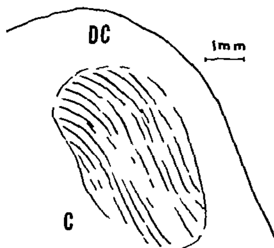


Fig. 13. Orientation of the dendritic laminae from the central nucleus, as seen in the frontal section show in Fig. 3

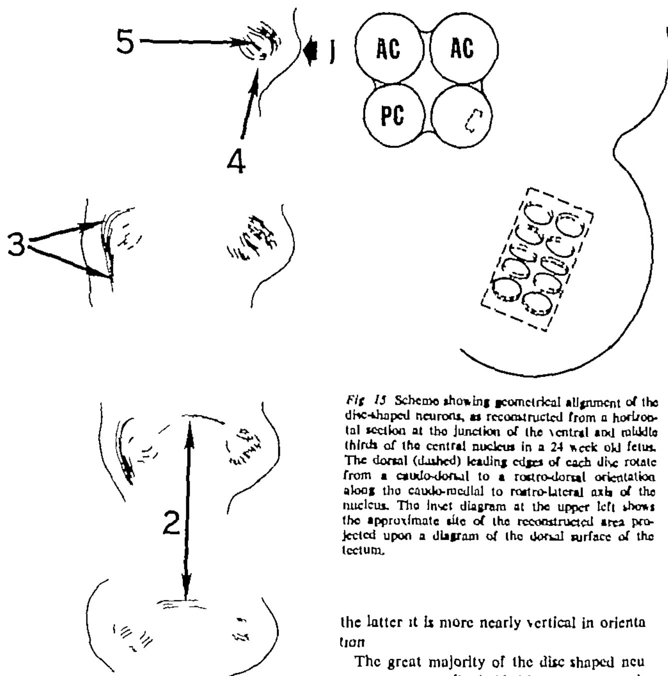


Fig 15 Scheme showing geometrical alignment of the disc-shaped neurons, as reconstructed from a horizontal section at the junction of the ventral and middle thirds of the central nucleus in a 24 week old fetus. The dorsal (dashed) leading edges of each disc rotate from a caudo-dorsal to a rostro-dorsal orientation along the caudo-medial to rostro-lateral axis of the nucleus. The inset diagram at the upper left shows the approximate site of the reconstructed area projected upon a diagram of the dorsal surface of the tectum.

Fig 14 Orientation of the dendritic laminae of the central nucleus (*5*) as seen in horizontal sections from a 24 week old fetus. In the right half of the figure the outlines from the right posterior colliculus appear at evenly spaced intervals in the central nucleus progressing from the ventral fourth at the top to the dorsal fourth at the bottom. In the left half of the figure are the outlines from the left posterior colliculus these correspond to more ventral levels than their partners because of the oblique plane of section *1* lateral edge of the right posterior colliculus, commissure of the posterior colliculus *3* axons of the lateral lemniscus, *4* caudo-central zone occupied by ascending lateral lemniscus *5* dendritic laminae of the central nucleus.

the latter it is more nearly vertical in orientation

The great majority of the disc shaped neurons appear equally divided between two main types, large and small (Fig 8) *The large disc shaped neuron* often has a dendritic field as long as one millimeter sometimes even longer (Fig 9) The dendrites are less elaborately branched than the other types in the central nucleus At the ventral, lateral and anterior boundaries of the nucleus, the dendrites often spread across the borders into the peri-collicular zones (Figs 19 *D* 28 *I* 30 *b*) but in the medial margin of the lateral zone their dendrites often bend to run in parallel with the boundary itself Dendritic growth cones evidence of late dendritic growth (Morest

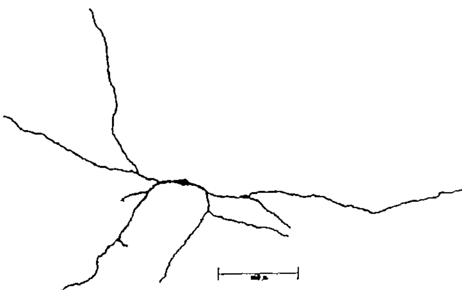


Fig. 16 Small stellate neuron, as seen in parasagittal section from the central nucleus of a 53 year old man. The dendrites in the long axis of the cell (from left to right) extend within the dendritic laminae in an antero-posterior direction. The dendrites projecting toward the lower left corner of the field extend in the horizontal plane within the dendritic laminae. The long dendritic branch pointing toward the top of the field transects the dendritic laminae in the dorso-ventral plane. Crosses at the tips of the processes in this and other figures indicate where they are transected at the surfaces of the sections.

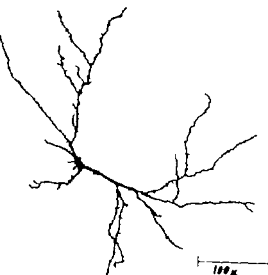


Fig. 17 Medium-sized stellate neuron, as seen in parasagittal section from the central nucleus of 53 year old man. The long axis of the dendritic field transects the plane of the dendritic laminae.

1969), appear commonly as they do on other cells in the posterior colliculus (Fig. 9). This occurs in adult as well as infant or fetal brains. The axon of the cell usually arises from the perikaryon. The initial direction of the axon varies and cannot be traced farther in these preparations. The long axis of this neuron is parallel to the incoming axons from the lateral lemniscus. *The small disc shaped neuron* has a dendritic field approximately 600 μ long (Fig. 10). It is mingled with large disc-shaped neurons, the dendrites of which branch in a similar pattern. However, the small cell has thinner dendrites with branches arranged in more or less discrete tufts. The usual course of their axons is uncertain, but in some cases, as the axons cross the laminae, they give off collaterals in nearby laminae (Fig. 10 *a*). This type of neuron is homologous to the small disc-shaped neurons described in the central nucleus of the posterior colliculus of the cat (Morest, 1964 *b*).

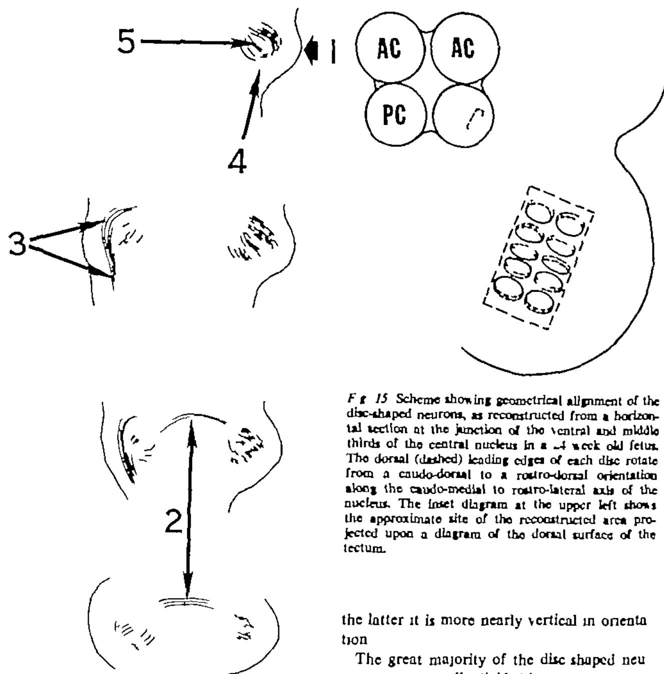


Fig 14 Orientation of the dendritic laminae of the central nucleus (*5*) as seen in horizontal sections from a 24 week old fetus. In the right half of the figure the outlines from the right posterior colliculus appear at evenly spaced intervals in the central nucleus, progressing from the ventral fourth at the top to the dorsal fourth at the bottom In the left half of the figure are the outlines from the left posterior colliculus, these correspond to more central levels than their partners because of the oblique plane of section. *1* lateral edge of the right posterior colliculus *2* commissure of the posterior colliculus, *3* axons of the lateral lemniscus, *4* caudo-ventral zone occupied by ascending lateral lemniscus, *5* dendritic laminae of the central nucleus.

Fig 15 Scheme showing geometrical alignment of the disc-shaped neurons, as reconstructed from a horizontal section at the junction of the ventral and middle thirds of the central nucleus in a 24 week old fetus. The dorsal (dashed) leading edges of each disc rotate from a caudo-dorsal to a rostro-dorsal orientation along the caudo-medial to rostro-lateral axis of the nucleus. The inset diagram at the upper left shows the approximate site of the reconstructed area projected upon a diagram of the dorsal surface of the tectum.

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The great majority of the disc shaped neurons appear equally divided between two main types, large and small (Fig. 8) *The large disc shaped neuron* often has a dendritic field as long as one millimeter sometimes even longer (Fig 9) The dendrites are less elaborately branched than the other types in the central nucleus At the ventral lateral and anterior boundaries of the nucleus, the dendrites often spread across the borders into the peri-collicular zones (Figs 19 *D* 28 *I* 30 *b*) but in the medial margin of the lateral zone their dendrites often bend to run in parallel with the boundary itself Dendritic growth cones, evidence of late dendritic growth (Morest,

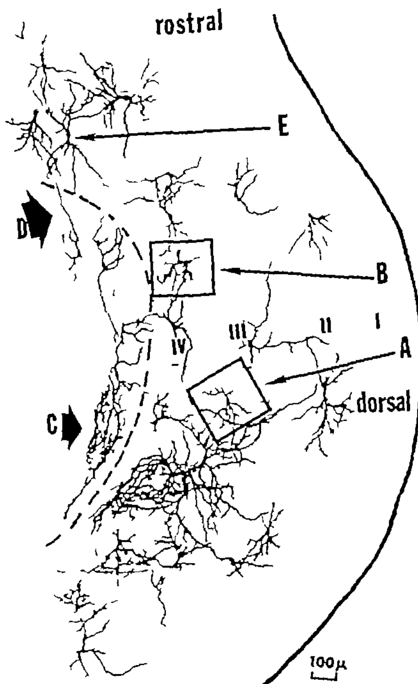


Fig. 19 The dorsal region of the cortex of the posterior colliculus in a paramagittal section at the junction of the medial and middle thirds of the posterior colliculus, *where the cortex is thickest. I II III IV* indicate the respective cortical layers. Layer I is not impregnated. *A* small thin-branched stellate neuron, which appears in both layers III and IV. *B* small thick-branched stellate neuron of the type that appears among layer IV neurons, especially in the caudal cortex at the caudal border of the central nucleus; *C* disc-shaped neurons of central nucleus; *D* neuron of the central nucleus with long dendrites extending beyond the rostral border of the nucleus; *E* large cell of the intercollicular or commissural zone of the posterior colliculus. 53 year old man.

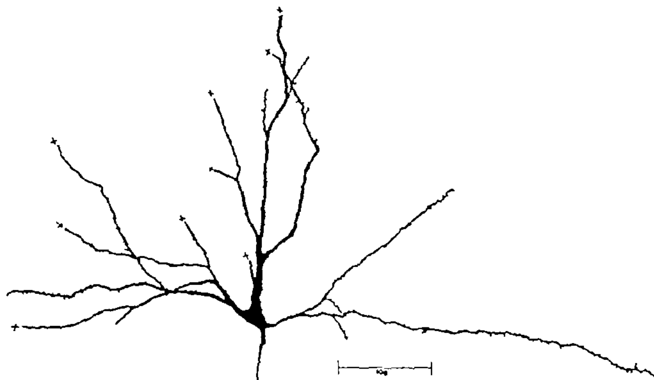


Fig 18 Large stellate neuron from a parasagittal section of the central nucleus of a 53 year old man. The dendrites cross the dendritic laminae at various angles. The thick initial segment of the axon projects from the perikaryon toward the bottom of the field.

The stellate cells appear to be less frequent than the disc shaped cells, although they appear throughout the central nucleus. At least three varieties of stellate cells can be distinguished small medium and large *The small stellate cell* is multipolar and has long thin dendrites, arranged in an elliptical field with a long axis of 600–800 μ (Fig. 16) The dendrites tend to collect in mutually perpendicular strands, either parallel or normal to the laminae The longest dendrites run parallel to the laminae and to the long axis of the disc shaped neurons Similar cells occur in the central nucleus of the cat's posterior colliculus (Morest, 1964 *b*) *The medium stellate cell* is a medium sized neuron, the dendrites of which radiate in a spheroidal field (Fig 17) The diameter of the dendritic field ranges from 600–800 μ its long axis crosses the dendritic laminae of the central nucleus at right angles. However some of the secondary branches may run parallel to the dendritic laminae Some dendritic branches may bend and travel with the incoming fibers of the central nucleus, while other branches may extend in various directions across or through the laminae Dendritic growth cones are evident *The large stellate cell* has dendrites, arranged in a spheroidal field, which cross the laminae in multiple directions (Fig 18) The diameter of the dendritic field usually approaches, and often exceeds, one millimeter This type of neuron appears to have a relatively low incidence. An analogous type of neuron has been observed in the central nucleus of the cat (Morest, 1964 *b*)

The cortex of the posterior colliculus contains different types and sizes of neurons, the bodies of which are situated in separate layers (Fig 7 DC) As in the cerebral cortex the dendrites of the neurons often cross more than one layer (Figs. 19 22) The cortex of the human posterior colliculus extends from the posterior end of the anterior colliculus and the dorsal tip of the intercollicular tegmentum over the dorsal convexity of the colliculus and onto the free posterior margin of the colliculus (the

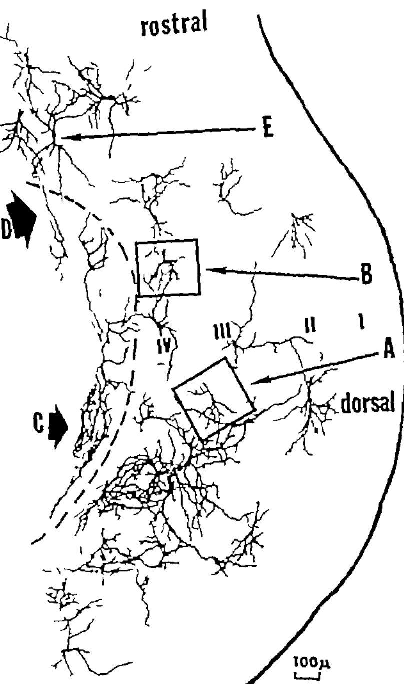


Fig. 19. The dorsal region of the cortex of the posterior colliculus in a parasagittal section at the junction of the medial and middle thirds of the posterior colliculus, where the cortex is thickest. *I, II, III, IV* indicate the respective cortical layers. Layer I is not impregnated. *A*, small thin-branched stellate neuron, which appears in both layers III and IV. *B*, small thick-branched stellate neuron of the type that appears among layer IV neurons, especially in the caudal cortex at the caudal border of the central nucleus. *C*, disc-shaped neurons of central nucleus; *D*, neuron of the central nucleus with a long dendrite extending beyond the rostral border of the nucleus; *E*, large cell of the intercollicular or commissural zone of the posterior colliculus. 53 year old man.

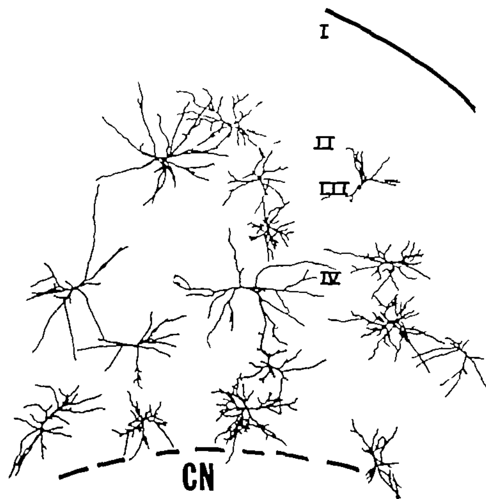


Fig 20 The dorsal cortex shown in a frontal section. Detail from Fig. 7 The neuron in the lower left corner of the field belongs to the part of the dorsomedial nucleus adjoining layer IV The medial direction is to the left Golgi-Cox, 55 year old man

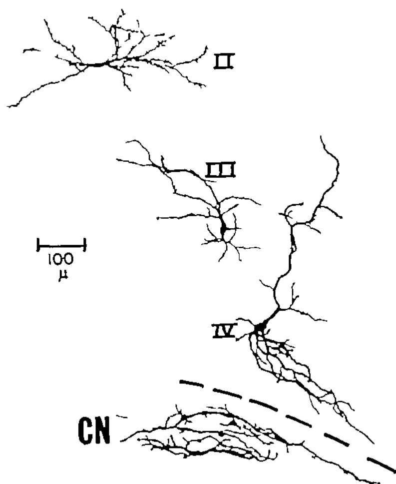


Fig 21 Dorsal cortex. Detail from Fig 19 showing the main cell types that distinguish layers II III and IV
A Initial segments of axons.

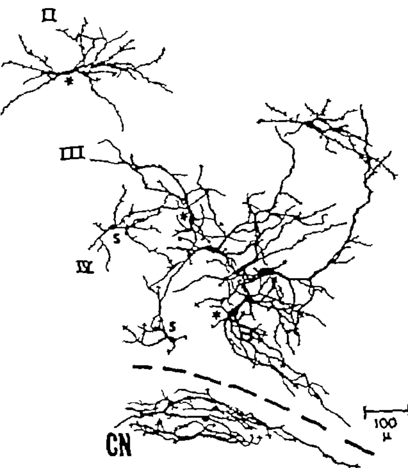


Fig. 22. Dorsal cortex. Same neurons ( ) as in preceding figure, but more examples are added to illustrate some of the overlap between different layers by the dendritic processes, and to show the small neurons ( ) that occur in both layers III and IV. *A*, a, initial segments of axons. The rostral direction is to the left.

caudal cortex) as far as the attachment of the anterior medullary velum and the decussation of the trochlear nerve (Figs. 4–6). Laterally the cortex gradually gives way to the lateral zone (Figs. 1–3, 7). In the human cortex, as in cats (Morest, 1966 *a*), four layers may be distinguished through most of its extent. However, in the caudal cortex and laterally the superficial layers attenuate greatly and eventually disappear posteroventrally. Also a fifth layer might be distinguished anteromedially in association with the fibers entering or leaving the commissure of the posterior colliculus (Figs. 7 *IC*, 19 *E*; see also Morest, 1966 *a*). In the present study we shall designate this last region, the intercollicular commissural zone. Generally in the cortex of the human posterior colliculus, as in the cat, larger neurons occur in successively deeper layers.

In layer I there are small neurons with radiating dendrites in flattened fields, oriented *parallel to the fiber capsule of the tectal convexity*. Because the impregnation of the margins of the present preparations is scanty, few neurons could be observed. Of course, their perikarya are readily demonstrable in Nissl-stained sections (Fig. 4). More centrally in *layer II* there is a small to medium-sized multipolar neuron (Figs. 19, 20). Its ovoid dendritic field, 600–800 μ in diameter, is aligned nearly parallel to the collicular surface (Figs. 21, 23). The axons leave the perikarya and take a course parallel to the convexity of the collicular surface. *Layer III* contains a medium-

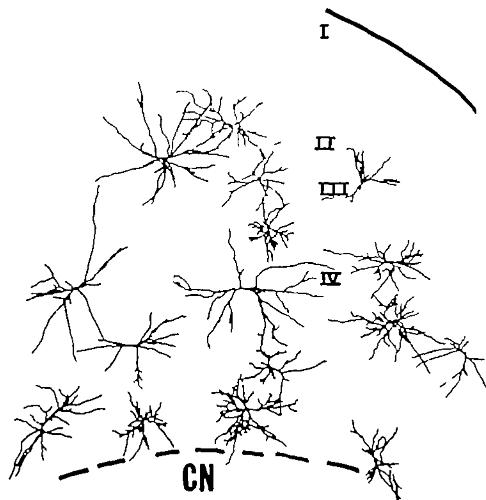


Fig 20 The dorsal cortex shown in a frontal section Detail from Fig. 7 The neuron in the lower left corner of the field belongs to the part of the dorsomedial nucleus *adjoining layer IV The* medial direction is to the left. Golgi-Cox, 55 year old man.

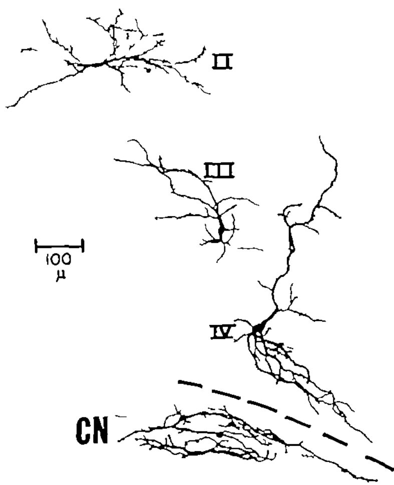


Fig 21 Dorsal cortex. Detail from Fig. 19 showing the main cell types that distinguish layers II, III, and IV
A Initial segments of axons.

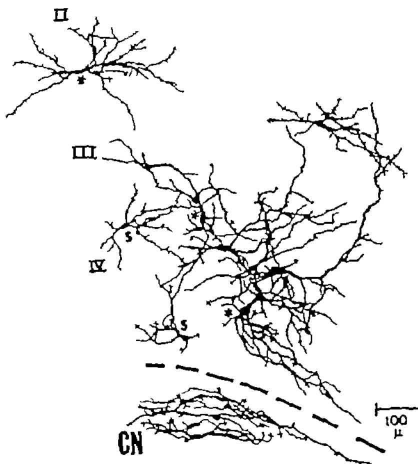


Fig. 22. Dorsal cortex. Same neurons ( ) as in preceding figure, but more examples are added to illustrate some of the overlap between different layers by the dendritic processes, and to show the small neurons (*s*) that occur in both layers III and IV *A* *a*, initial segments of axons. The rostral direction is to the left.

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In layer I there are small neurons with radiating dendrites in flattened fields, oriented parallel to the fiber capsule of the tectal convexity Because the impregnation of the margins of the present preparations is scanty few neurons could be observed. Of course, their perikarya are readily demonstrable in Nissl-stained sections (Fig. 24) More centrally in *layer II* there is a small to medium-sized multipolar neuron (Figs. 19 20). Its ovoid dendritic field, 600–800 µ in diameter is aligned nearly parallel to the collicular surface (Figs. 21 23) The axons leave the perikarya and take a *course parallel to the* convexity of the collicular surface. *Layer III* contains a medium

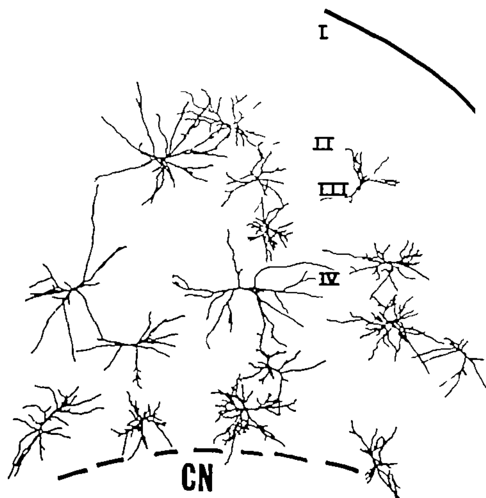


Fig 20 The dorsal cortex shown in a frontal section. Detail from Fig. 7 The neuron in the lower left corner of the field belongs to the part of the dorsomedial nucleus adjoining layer IV The medial direction is to the left. Golgi-Cox 55 year old man.

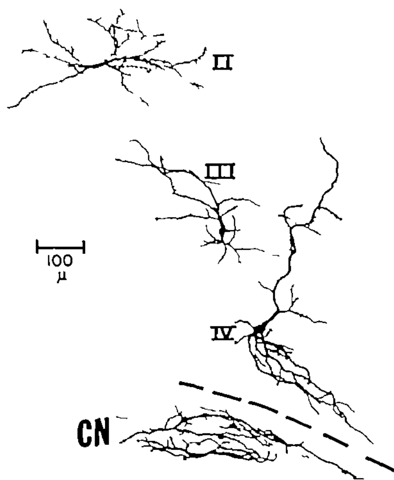


Fig 21 Dorsal cortex. Detail from Fig 19 showing the main cell types that distinguish layers II III and IV *A* initial segments of axons.



Fig. 25. Dorsal cortex. Neurons from layer III. the upper cell is small multipolar neuron with radiating branches arranged in a spheroidal field, the lower cell is a medium-sized neuron with dendrites branching in the form of bouquets. *a*, initial segment of axon. Golgi-Cox, parasagittal section, 53 year old man.

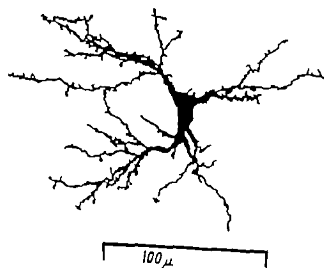


Fig. 26. Dorsal cortex, layer IV small multipolar neuron. Note club-shaped dendritic appendages and richer dendritic branching, compared to small neurons of layer III shown in preceding figure. Golgi-Cox, parasagittal section, 53 year old man.

Fig 23 Dorsal cortex in a parasagittal section through its central portion. *1* typical multipolar neuron of layer II *2 3 4* large multipolar neurons in the superficial region of layer IV which extend long dendrites across the more superficial layers, sometimes as far as layer I (*3*) and send other dendrites parallel to the layers. *5 6* large multipolar neurons of layer IV with dendrites extending in various directions in spherical or ovoid fields. *7* large multipolar neurons with dendrites elongated horizontally in layer IV The rostral direction is to the left Golgi-Cox.

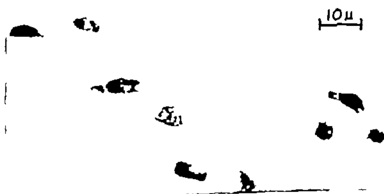


Fig 24 Dorsal cortex, layer I small neurons with a pale vesicular nucleus, containing a prominent nucleolus and slightly elongated cell body with its long axis parallel to the surface of the colliculus. Transverse section, cresyl violet. Adult

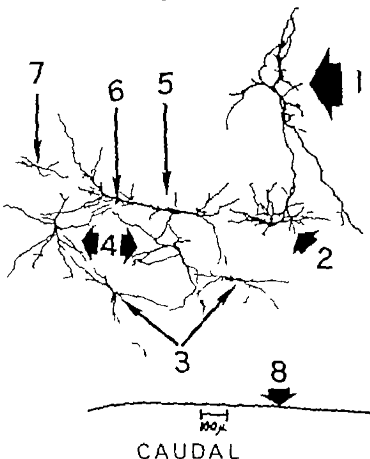


Fig. 28. Caudal cortex, parasagittal section adjacent to that of the preceding drawing. The ventral direction is to the left. *1* large disc-shaped neuron of the central nucleus with two distal dendritic processes extending into layer IV of the caudal cortex. *2* large multipolar neuron of layer IV with some dendrites extending along the boundary with the central nucleus and others directed toward the central nucleus. *3* medium-sized multipolar neurons of layer III. *4* large multipolar neurons of layer IV. *5* large elongated neuron of layer IV extending along the boundary of the central nucleus. *6 7* small multipolar neurons of layer IV with delicate dendrites. *8* pia mater. Golgi-Cox, 53 year old man.

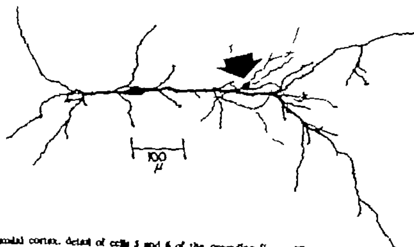


Fig. 29. Caudal cortex, detail of cells 5 and 6 of the preceding figure. The arrow indicates cell 6.

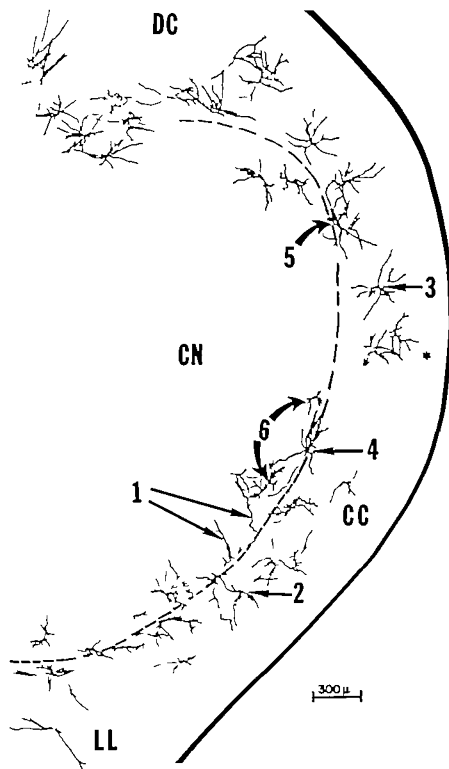


Fig 27 Dorsal and caudal cortex, parasagittal section through junction of middle and lateral thirds of posterior colliculus showing the transition ( ) from dorsal to caudal cortex. *1* dendritic processes of the central nucleus, oriented parallel to the dendritic laminae, point caudally toward the caudal cortex. *2* neuron of layer III of the caudal cortex. *3* neuron of layer III of the dorsal cortex. *4* large neuron of layer IV of the caudal cortex with a dendritic extension into the central nucleus. *5* neuron similar to the preceding in layer IV of the dorsal cortex. *6* medium sized multipolar neurons of the central nucleus with spheroidal dendritic fields: the dendrites tend to line up parallel to the border of the central nucleus but perpendicular to the laminae of the central nucleus. Golgi-Cox, 53 year old man

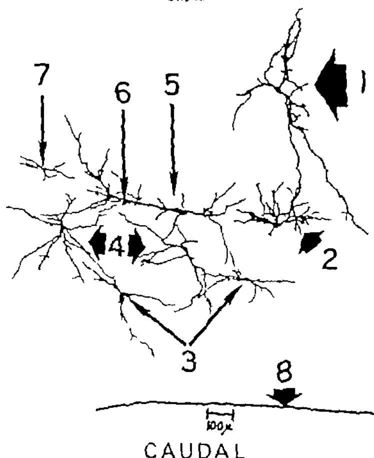


Fig. 28. Caudal cortex; parasagittal section adjacent to that of the preceding drawing. The ventral direction is to the left. *1* large disc-shaped neuron of the central nucleus with its distal dendritic processes extending into layer IV of the caudal cortex. *2*, large multipolar neuron of layer IV with some dendrites extending along the boundary with the central nucleus and others directed toward the central nucleus. *3* medium-sized multipolar neurons of layer III. *4* large multipolar neurons of layer IV *5* large elongated neuron of layer IV extending along the boundary of the central nucleus. *6*, *7* small multipolar neurons of layer IV with delicate dendrites. *8* pia mater Golgi-Cox, 53 year old man.

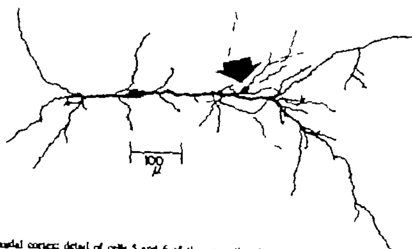


Fig. 29 Caudal cortex: detail of cells 5 and 6 of the preceding figure. The arrow indicates cell 6.

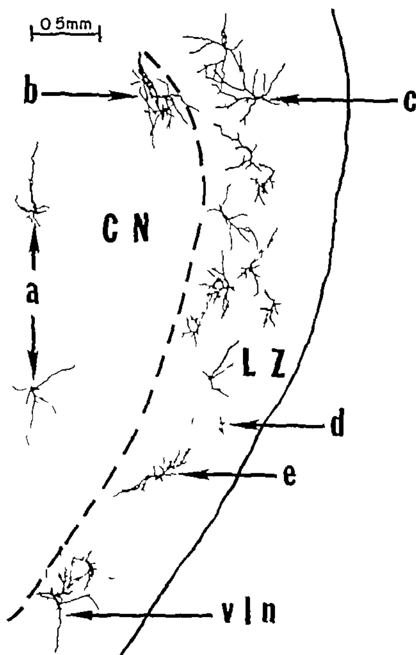


Fig 30 Lateral zone (*LZ*) and ventrolateral nucleus (*Vln*): detail from Fig. 7 *a* disc shaped neurons of the central nucleus (*CN*), *b* neurons of the central nucleus at its dorsolateral margin, *c* neuron of layer III of the dorsal cortex adjacent to the lateral zone *d* small, thin-branched multipolar neuron, embedded among the fibers of the brachium of the posterior colliculus, *e* large multipolar neurons of the lateral zone which resemble the large neurons of layer IV of the dorsal cortex.

sized multipolar neuron the dendrites of which occupy a spheroidal field, some 600–800 μ in diameter (Fig 21) There is a characteristi branching pattern in which the secondary dendrites form in bouquets at the major branching points (Fig 25) A few of the dendrites may extend parallel to the collicular surface while others run perpendicularly some as far as the adjacent layers (Figs. 19 27 *2 3* 28 *3*) The axon cannot be traced beyond the initial segment A smaller type of neuron with thin radiating dendrites about 200 μ long also occurs in layer III (Fig 25) The short dendrites do not extend into the neighboring layers (Fig 19 *A*) Such neurons also appear in *layer IV* (Figs 22, *S* 28 *6 7* 29) Other small neurons in layer IV have slightly coarser and more richly branched dendrites (Figs 19 *B* 26) these cells are prominent at the caudal border of the central nucleus More characteristic of layer IV however is the large multipolar neuron with a dendritic field diameter of 800–1 000 μ (Fig 21) The dendritic arrangement varies according to its location in the layer (Fig 20) The most superficial neurons send long dendrites through layer III to

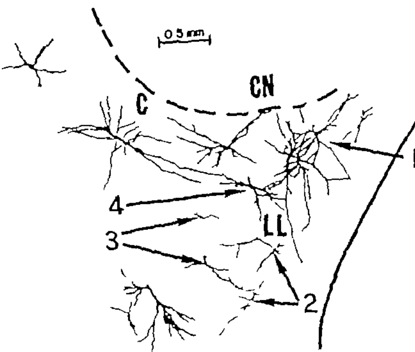


Fig. 31 Dorsal nucleus of the lateral lemniscus (*LL*) and cuneiform area (*C*): detail from *Fig. 7*. *1* ventral-most neurons of the centrolateral nucleus. *2* small-to-medium-sized discoid neurons, oriented with their long axes parallel to the fibers of the lateral lemniscus. *3* small-to-medium-sized discoid neurons with their long axes perpendicular to the fibers of the lateral lemniscus. *4* large stellate neuron of the dorsal nucleus of the lateral lemniscus with its dendrites radiating obliquely across the fibers of the lateral lemniscus.

end in layers I or II (Fig. 23, *2 3 4*). More deeply situated neurons, nearer the central nucleus, often have dendrites coursing parallel to the margin of the central nucleus (Figs. 23 *7* 28, *5* 29) Sometimes two or more dendrites from the same neuron collect, as if in a fascicle, and extend parallel to the margin of the central nucleus in association with axons taking the same path. Less frequently the neurons very near the margin of the central nucleus have spheroidal or oval dendritic fields (Fig. 23 *5 6*). Any of the deeply situated neurons may send dendrites into the peripheral edge of the central nucleus (Figs. 27 *4 5* 28, *2*) or among the most superficial neurons of layer IV (Fig. 22). The largest neurons tend to occur in the middle of layer IV. Their dendrites, extending in various directions in a spheroidal field, often reach the more superficially and deeply situated cells of layer IV (Figs. 22, 20).

The commissural zone contains two basic types of neurons (Fig. 7). More superficially just beneath the unimpregnated margin of the commissural zone are small, highly branched cells, occupying a spheroidal field 400–500 μ in diameter. Frequently one dendrite is more highly branched than the others, as in a bouquet or tuft (Fig. 33 bottom). Some dendrites parallel the paths of the commissural fibers, while other dendrites cross the fibers at right or oblique angles. The axon arises from the perikaryon. These cells might be properly regarded as an interstitial nucleus of the commissure. Peripheral dendritic *growth cones* and dendritic processes, apparently just sprouting from the cell body occur on these cells at all of the ages examined.

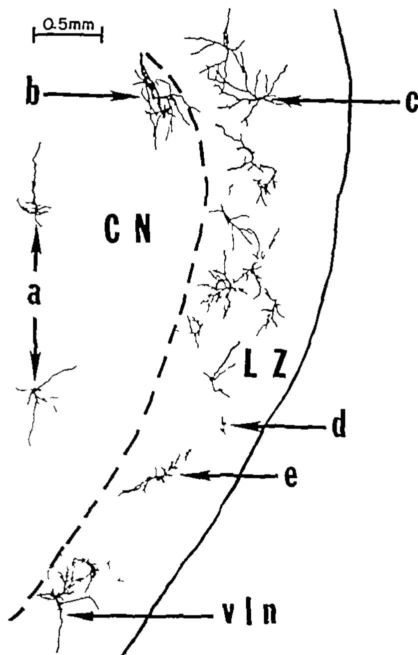


Fig 30 Lateral zone (*LZ*) and ventrolateral nucleus (*Vln*) detail from Fig. 7 *a* disc-shaped neurons of the central nucleus (CN), *b* neurons of the central nucleus at its dorsolateral margin, *c* neuron of layer III of the dorsal cortex adjacent to the lateral zone, *d* small, thin-branched multipolar neuron, embedded among the fibers of the brachium of the posterior colliculus, *e* large multipolar neurons of the lateral zone which resemble the large neurons of layer IV of the dorsal cortex.

sized multipolar neuron the dendrites of which occupy a spheroidal field some 600–800 μ in diameter (Fig. 21) There is a characteristic branching pattern in which the secondary dendrites form in bouquets at the major branching points (Fig 25) A few of the dendrites may extend parallel to the collicular surface while others run perpendicularly some as far as the adjacent layers (Figs. 19 27 *2 3* 28, *3*) The axon cannot be traced beyond the initial segment. A smaller type of neuron with thin, radiating dendrites about 200 μ long also occurs in layer III (Fig 25) The short dendrites do not extend into the neighboring layers (Fig 19 *A*) Such neurons also appear in *layer IV* (Figs 22, *S* 28 *6 7* 29) Other small neurons in layer IV have slightly coarser and more richly branched dendrites (Figs. 19 *B* 26) these cells are prominent at the caudal border of the central nucleus. More characteristic of layer IV however is the large multipolar neuron with a dendritic field diameter of 800–1 000 μ (Fig 21) The dendritic arrangement varies according to its location in the layer (Fig 20) The most superficial neurons send long dendrites through layer III to

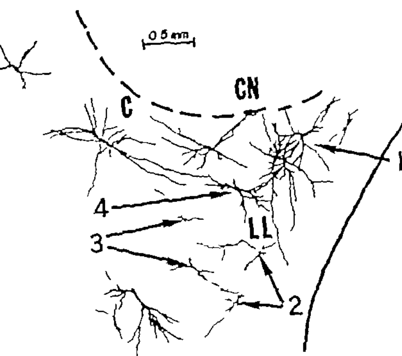


Fig. 31. Dorsal nucleus of the lateral lemniscus (*LL*) and cuneiform area (*C*), detail from Fig. 7. *1.* extralemnal neurons of the ventrolateral nucleus. *2.* small-to-medium-sized discoid neurons, oriented with their long axes parallel to the fibers of the lateral lemniscus. *3.* small-to-medium-sized discoid neurons with their long axes perpendicular to the fibers of the lateral lemniscus. *4.* large stellate neuron of the dorsal nucleus of the lateral lemniscus with its dendrites radiating obliquely across the fibers of the lateral lemniscus.

end in layers I or II (Fig. 23, 2, 3, 4). More deeply situated neurons, nearer the central nucleus, often have dendrites coursing parallel to the margin of the central nucleus (Figs. 23, 7, 28, 5, 29). Sometimes two or more dendrites from the same neuron collect, as if in a fascicle, and extend parallel to the margin of the central nucleus in association with axons taking the same path. Less frequently the neurons very near the margin of the central nucleus have spheroidal or oval dendritic fields (Fig. 23, 5, 6). Any of the deeply situated neurons may send dendrites into the peripheral edge of the central nucleus (Figs. 27, 4, 5, 28, 2) or among the most superficial neurons of layer IV (Fig. 22). The largest neurons tend to occur in the middle of layer IV. Their dendrites, extending in various directions in a spheroidal field, often reach the more superficially and deeply situated cells of layer IV (Figs. 22, 20).

The commissural zone contains two basic types of neurons (Fig. 7). More superficially, just beneath the unimpregnated margin of the commissural zone, are small, highly branched cells, occupying a spheroidal field, 400–500 μ in diameter. Frequently one dendrite is more highly branched than the others, as in a bouquet or tuft (Fig. 33, bottom). Some dendrites parallel the paths of the commissural fibers, while other dendrites cross the fibers at right or oblique angles. The axon arises from the perikaryon. These cells might be properly regarded as an interstitial nucleus of the commissure. Peripheral dendritic growth cones and dendritic processes, apparently just sprouting from the cell body, occur on these cells at all of the ages examined.

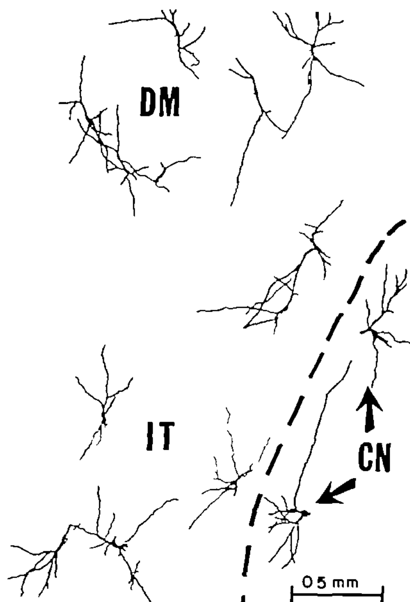


Fig 3 Dorsomedial nucleus (*DM*) and medial intercollicular tegmentum (*IT*): detail from Fig. 7

In Nissl stained sections there is a small group of neurons in the midline overriding the superficial strands of the commissure (Fig 2) This nucleus is not impregnated in the present preparations (Fig 3) although it has been observed in Golgi impregnations of the cat

The larger neurons in the commissural zone occupy more ventral or medial positions (Fig 7) This type of cell, with a dendritic field of 500–600 μ in diameter has 4–6 major branching points (Fig 19 *E*) One or two dendrites usually are parallel to the commissural fibers, while the remaining dendritic branches cross the commissural fibers at right angles. The longest dendrite appears somewhat thicker than the rest and usually parallels the commissural fiber path. Each dendrite has only one or two secondary branches, undergoes little tapering and has a fluffy appearance, due to many small appendages. The axon takes off in a dorsal direction The most ventral cells (Fig 3, top) resemble the neurons typically found in the peri-collicular tegmentum

Peri-collicular tegmentum The neurons of the midbrain tegmentum, e g., the small-to-mediumsized neurons of the *cuneiform area* are characterized by relatively few long, slender straight infrequently branched dendrites usually without prominent spines or append-

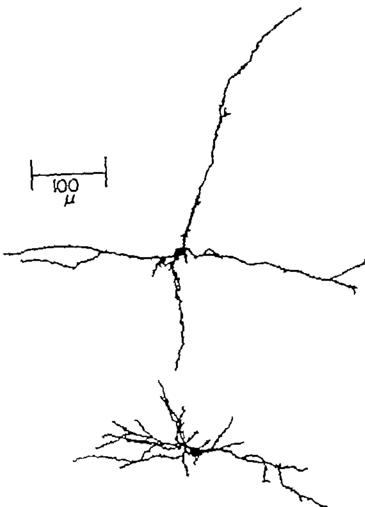


Fig. 31. Intercollicular area associated with the commissure of the posterior colliculus from Fig. 7. Large (top) and small (bottom) types of neurons. Note the terminal dendritic growth cones at the tip of the right-hand dendrite of the large neuron and at the incipient branch half-way along the upper dendrite. Golgi-Cox, *frontal section, 55 year old man.*

ages, whereas the tectal neurons typically have many more dendrites, which are shorter more highly branched, and more compactly arranged (Fig. 7). Since the commissural zone *contains neurons resembling the tegmental* type to some extent this zone could even be regarded as a tegmental region or perhaps a transitional region between tectum and tegmentum. Likewise the regions just medial and just lateral to the central nucleus contain some neurons that are more typically tegmental in form and some that are more typically tectal. Nevertheless, we have included these pericollicular groups in the *tegmentum*, since, unlike the commissural zone, they receive projections from the spinal cord in the cat and opossum. (Morest, 1966 *b*).

The intercollicular tegmentum appears as a thin strip of tegmental neurons extending *anterodorsally from the cuneiform area* between the medial aspect of the central nucleus and the central gray (Fig. 7). Anteriorly it extends to the roof of the midbrain as a thin *tongue of tegmentum, separating the anterior* from the posterior colliculus (Fig. 6). It contains small neurons, similar in form to those of the cuneiform area (Fig. 32).

The dorsomedial nucleus appears to be a specialized aggregation of the medial intercollicular tegmentum, wedged in the angle

formed by the central nucleus, dorsal cortex commissural zone and central gray (Fig 7) The neurons of the dorsomedial nucleus resemble tegmental cells but have thinner more delicate dendrites with more branches than those of the adjacent tegmentum (Fig 32) This nucleus seems to stand out from the rest of the intercollicular zone by virtue of the greater packing density of its cells and the more compact arrangement of their processes

The lateral zone of the peri-collicular tegmentum resembles the intercollicular tegmentum in that it contains many small neurons of the tegmental type which appear to be an anterolateral extension of the cuneiform area (Fig 7) Anteriorly the lateral tegmental cells merge with the intercollicular tegmentum curving around the anteromedial aspect of the central nucleus, so that in a sense, the tegmental type of neurons in the lateral zone could be regarded as the lateral intercollicular tegmentum The lateral zone occupies the lateral surface of the posterior colliculus (Fig. 30) It extends from the dorsal nucleus of the lateral lemniscus and *pedamentum saguli*, ventrally and posterolaterally to the dorsal cortex and brachium of the posterior colliculus, dorsally and anterolaterally The fibers of the brachium gather in the margin of the lateral zone, where its cells merge with the small multipolar neurons of the interstitial nucleus of the brachium (Fig 30 *d*) Many of the neurons, especially the largest ones (Fig 30 *e*) have a more distinctly tectal appearance by virtue of their more elaborate dendritic branching pattern The large neurons resemble the large cells of layer IV in the collicular cortex but the latter tend to be smaller and to have thinner more richly branched dendrites Perhaps the large neurons of the lateral zone could be regarded as derivatives of the fourth cortical layer

The ventrolateral nucleus (Fig 7 *vln*) on the ventrolateral border of the central nucleus in the lateral zone is the counterpart of the dorsomedial nucleus in the medial intercollicular tegmentum However the ventrolateral nucleus is conspicuous for its content of very large neurons—the largest in the lateral zone—and smaller ones of tegmental type (Figs. 30 *vln* 31 *1*)

The dorsal nucleus of the lateral lemniscus (Fig 7 *LL*) contains large, medium and small neurons with sparsely branched dendrites, resembling their neighbors in the cuneiform area But the dendritic branching patterns of the dorsal nucleus are more tightly drawn (Fig 31) The small neuron has a flattened dendritic field oriented with its long axis parallel to the fibers of the lateral lemniscus (Fig 31 *2*) whereas the medium sized neuron has a flat dendritic field, arranged perpendicular to the lemniscal fibers (Fig 31 *3*) The large neuron has a stellate dendritic field, radiating obliquely across the lemniscus (Fig 31 *4*)

Discussion

The present study appears to be the first detailed analysis of the neuronal architecture of the human posterior colliculus. The distinction of a central nucleus and a rind or cortex in the mammalian posterior colliculus generally agrees with previous histological accounts with the Golgi method in the cat, dog mouse, and opossum (Ramón y Cajal 1911 Morest, 1964 *b* 1966 *a*, *b* 1969)

The significance of the present findings derives from the dendritic morphology of the constituent neurons. These cells appear to be so stereotyped in the size, shape and branching pattern of their dendrites that they are readily described as populations of fixed morphological types of neurons Thus as in the medial geniculate body (Morest 1964 *a*) one may determine the extent of the typical populations

n greater precision and with considerably re accuracy than would have been possible h the standard methods of Nissl and Wei-t alone. When the observations are com red in detail with those in the cat (Morest, 64 *b* 1966 *a* *b*) and opossum (Morest, 1969), is possible to identify homologous popula-ns of neurons, according to the principles plied to the medial geniculate body (Morest, 965 c). This is possible because the stereo-yped dendritic branching patterns of different neuronal types are relatively consistent in the several mammalian species examined and, in this respect, appear to be conservative in phylogeny Moreover the embryological observations of the temporal sequence and spatial pattern of neurogenesis in the central nucleus and cortex are comparable in the opossum and cat. *Consequently having critically identified the homologous neurons in the cat we may now hope to apply what little is known of the anatomy and physiology of these species to the human auditory system. Further more since the dendrites of neurons generally accommodate the overwhelming preponderance of their synaptic contacts the geometrical properties of the dendrites alone can define certain aspects of central auditory function.*

The cent al nucleus appears to be homologous to the same nucleus previously identified in the cat (Ramón y Cajal, 1911 Morest, 1964 *b*). In both species the large and small disc-shaped neurons and the varieties of stellate cells are quite comparable However in the cat the large disc shaped neurons tend to congregate in the lateral parts of the central nucleus (Morest, 1966 *b*) Neurons with short axons (Golgi type II), as seen in the cat, have not yet been definitely identified in the present material, although Held (1893) and Kölliker (1896) indicate their existence in the human posterior colliculus. The technique used in the present study is not well suited for their impregnation. In the cat, it was proposed that the laminar arrangement of the disc-shaped neurons in parallel with the ascending auditory afferents would provide the optimum basis for preservation of the tonotopic organization of the auditory pathway (Morest, 1964 *c*) This probably agrees with the microelectrode recordings of the sequence of best frequencies of units found in the central nucleus of the cat (Rose Greenwood, Goldberg & Hind 1963) Similar geometric constellations have been correlated with the tonotopic organiza tion of the auditory system in the medial geniculate body (Morest, 1965 *a*) and in the trapezoid and superior olivary nuclei (Morest, 1968). The probable significance of the stellate neurons is less clear In the cat the axons of the stellate neurons have extensively branched collaterals that reach wide sectors of the fibro-dendritic laminae. The elongated variety that extends perpendicularly across the laminae would intercept a wide sector of the afferent fiber spectrum. Hence it might be expected to have a very broad tuning curve. Such an arrangement would favor detection or summation or an averaging of signals over wide segments of the afferent fiber spectrum The stellate neurons could exert widespread influences, inhibitory or excitatory on the indigenous activity of the nucleus. Whether they might be involved in binaural interaction, the summation of stimulus intensities, or regulation of the general levels of spontaneous activity are matters for speculation. It is not certain which types of neurons project to the medial geniculate body although a projection from the disc-shaped cells could account for a colliculo-geniculate correspondence of the tonotopic organization.

The cortex of the posterior colliculus in man and cat is characterized by flat, elongated neurons in the most superficial layer and stellate neurons in deeper layers that are concentric to the convexity of the colliculus. Since the perikarya in successively deeper layers are progressively larger the layers may be delineated in transverse, Nissl- or silver-stained sections. In Golgi preparations homologous types of neurons appear in the corresponding layers of both cat and man. This cortex is

formed by the central nucleus, dorsal cortex commissural zone and central gray (Fig 7) The neurons of the dorsomedial nucleus resemble tegmental cells but have thinner more delicate dendrites with more branches than those of the adjacent tegmentum (Fig 32) This nucleus seems to stand out from the rest of the intercollicular zone by virtue of the greater packing density of its cells and the more compact arrangement of their processes

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neuronal morphology. In the cat these nuclei are the principal collicular termini of the descending pathway from the auditory cortex (Rasmussen, 1964). Since these nuclei are well represented in the human, one may suppose that the descending auditory pathway is also well represented. However no indications of their possible functions may be given, until their efferent connections and some relevant electrophysiological data are available.

The typical neurons of the peri-collicular tegmentum that appear medial to the central nucleus and in the lateral zone, including the medial and lateral intercollicular tegmentum, respectively correspond to similar groups of neurons observed in the cat (Morest, 1966 *b*). These regions of the posterior colliculus are remarkable because they receive projections from the spinal cord and, in the case of the lateral zone from the dorsal column nuclei (Morest, 1966 *b*). Indeed, Campbell (1944) has recorded evoked somatic sensory potentials from a region that may correspond to the lateral zone. These regions also receive axonal collaterals from the auditory pathways. Probably the medial and lateral tegmental regions are extensions of the cuneiform area and lateral mesencephalic tegmentum, which exhibit similar neuronal morphology and similar afferent connections (Morest, 1965 *b*). All of these tegmental regions may provide for the integration of somatic sensory and auditory inputs at the unit level (Huttenlocher 1960). Moreover these regions probably contribute to the lateral tegmental system of the midbrain, which contains the main ascending input to the dorsal division of the medial geniculate body and the rest of the posterior group (Morest, 1965 *b*) where there is electrophysiological evidence for integration of the somatic sensory and auditory inputs by individual neurons (Poggio & Mountcastle, 1960).

The dendritic growth cones commonly observed in the human posterior colliculus may be construed as morphological signs of growth and neuronal plasticity in both the immature and adult brain. These characteristic features have been correlated with the growth activity of dendrites in developing brains (Morest, 1969). In a number of cases dendritic growth cones have been observed in populations of otherwise mature neurons from young adult brains in the cat and opossum (Morest, 1969, 1971). We now report their common occurrence in aged as well as young humans. Our impression is that the dendritic growth cones are rather more common and widespread in the mature posterior colliculus of the present human material than of the opossum. It is not possible at present to decide whether the dendritic growth in mature and aged individuals reflects a continuation of development, perhaps even related to learning processes, or represents a dystrophic reaction to a degenerative process.

Summary

This report gives the detailed analysis of the neuronal architecture of the human posterior colliculus (auditory tectum). The analysis is made on Golgi-Cox impregnations of midbrains from autopsy cases, ranging from 24 weeks of gestation to 74 years of age. The neurons of the posterior colliculus are so stereotyped in the relative sizes, shapes, and branching patterns of their dendrites that they are readily defined as distinctive morphological populations arranged in specific geometrical constellations, namely the central nucleus, the collicular cortex, and the nuclei of the pericollicular tegmentum. Because these morphological features are relatively stable in mammalian phylogeny and embryogenesis, it is possible to identify the homologous populations of neurons in the cat. Thus we may now begin to relate to the human the anatomical and physiological analyses of the homologous

indeed a cortical structure, in the general sense of the term as applied to the marginal gray matter of the cerebrum cerebellum and olfactory bulb In all of these cortices morphologically distinct layers of the neuropil are intercepted to different extents by the various dendrites crossing them and the layers are interconnected. Cytoarchitectonic studies have confused the cortex of the posterior colliculus with the posterior extent of the anterior colliculus (e g Huber & Crosby 1943) or have apparently relegated it to a narrow strip of tissue associated with the myelinated fiber capsule covering the surface of the tectum (e g Kölliker 1896 Winkler 1921 Riley 1943 Berman 1968 Van Noort [2] 1969) or have ignored it (e g Held 1893 Dejerine 1895 Valeton, 1908 Sterzi 1915 Olszewski & Baxter 1954) The present findings agree with the very brief account of the posterior colliculus given by Ramón y Cajal (1911) in small mammals. In fact, the cortex occupies a large portion of the posterior colliculus Although the relative proportions of the cortex and central nucleus may be comparable in man and cat the cortex appears much smaller in the opossum The cortex is sharply distinguished from the anterior colliculus and neighboring regions by its neuronal architecture and by different afferent connections as well (Morest 1966 a b)

The afferent connections of the cortex of the posterior colliculus are not completely known (see Morest, 1966 a) In the cat auditory axons, ascending primarily to the central nucleus send collaterals to layer IV along with the collaterals of the efferent axons of the central nucleus Thus layer IV is in a position to integrate elements of both the input and output of the central nucleus. The superficial layers receive projections from more anterior levels of the brain The neurons of layer III are best situated to integrate elements of both the ascending and descending pathways, especially by way of intrinsic connections of the neurons of layers II and IV which receive the heaviest exogenous projections. The pattern of intrinsic connections would permit layer III to monitor input and output activity in all other layers Thus the patterns of neural activity that can be generated by the cortex are limited by the spatial patterns of its intrinsic organization How these patterns may relate the posterior colliculus to functions of the auditory system such as the acoustic reflexes or the integration of the ascending and descending auditory pathways, must depend in part on the details of the efferent projections of the posterior collicular cortex (Morest, 1966 a, unpublished)

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The typical neurons of the peri-collicular tegmentum that appear medial to the central nucleus and in the lateral zone, including the medial and lateral intercollicular tegmentum, respectively correspond to similar groups of neurons observed in the cat (Morest, 1966 *b*). These regions of the posterior colliculus are remarkable because they receive projections from the spinal cord and, in the case of the lateral zone, from the dorsal column nuclei (Morest, 1966 *b*). Indeed, Campbell (1944) has recorded evoked somatic sensory potentials from a region that may correspond to the lateral zone. These regions also receive axonal collaterals from the auditory pathways. Probably the medial and lateral tegmental regions are extensions of the cuneiform area and lateral mesencephalic tegmentum, which exhibit similar neuronal morphology and similar afferent connections (Morest, 1965 *b*) All of these tegmental regions may provide for the integration of somatic sensory and auditory inputs at the unit level (Huttenlocher 1960) Moreover these regions probably contribute to the lateral tegmental system of the midbrain, which contains the main ascending input to the dorsal division of the medial geniculate body and the rest of the posterior group (Morest, 1965 *b*) where there is electrophysiological evidence for integration of the somatic sensory and auditory inputs by individual neurons (Poggio & Mountcastle, 1960)

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structures of the cat Furthermore since the dendrites of neurons accommodate the preponderance of their synaptic contacts the geometrical properties of the dendrites must define certain aspects of central auditory function The central nucleus contains neurons *with disc shaped dendritic fields* arranged in parallel layers that interdigitate with the ascending auditory axons Such an arrangement provides the optimum basis for preservation of the tonotopic organization of the ascending auditory pathway Stellate neurons intercept the fibro-dendritic layers at right angles, so as to foster a more widespread reaction with the afferent auditory spectrum and may be involved in binaural or amplitude distinctions. The *collicular* cortex consists of distinct layers of neurons, which differ morphologically but which interconnect the layers by their dendrites and axons The intrinsic organization of the collicular cortex and its relationships to the collaterals of extrinsic afferent fibers would permit it to monitor the inputs and outputs of the *ascending* and descending auditory pathways of the midbrain, which are perhaps involved in the integration of acoustic reflexes. The neuronal groups of the peri-collicular tegmentum may *provide for interactions of* the auditory and somatic sensory pathways, with which they are connected by way of the ascending sensory lemnisci and descending corticotectal and tecto-reticular tracts The dendritic growth cones commonly observed in the posterior colliculus provide morphological evidence of growth and neuronal plasticity in the brains of immature, adult and aged humans The anatomical findings have significance for the functional interpretation of pathological and behavioral studies of the central auditory system

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SUPPLEMENT 296

Development of the Middle and Inner Ear in the Golden Hamster (*Mesocricetus auratus*)

A Detailed Description to Establish A Norm for Physiopathological Study of Congenital Deafness

BY

CHARLENE B. STEPHENS, PH.D



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I Introduction

Approximately 8 500 000 Americans have either bilateral or unilateral hearing impairments of handicapping magnitude. There are approximately 40 000 deaf individuals between the ages of 5 and 18 years in the United States, a number representing a prevalence of about 88 per 100 000 children in this age range. Most deafness is congenital or is acquired relatively early in life (Carhart, 1969).

The goal of this study is to develop a laboratory animal for the study of congenital deafness. The golden hamster possesses features that make it a desirable animal for future research. These include a gestation period of 16 days, ease of obtaining timed matings, large litters, and susceptibility to certain drugs and viruses (Ferm, 1965 1967).

Many leading authors in otologic research and programmatic statements from authoritative public agencies, e g. National Institute of Neurological Diseases and Stroke and The Deafness Research Foundation, have emphatically stressed the dire need for more information in many areas basic to otologic practice These research needs include investigations in the embryological development of the inner ear fine anatomical detail with electron microscopy and cochlear pathology In specific demand should be ear specimens that are identified with sensori-neural deafness, the sequelae of viral infections, rubella, mumps-induced deafness, congenital deafness, cochlear otosclerosis, Menière's disease, and noise-induced nerve deafness. Many advances have been made in middle ear surgery for the relief of diseases of the conductive mechanism. In contrast, practically nothing is known for treating inner ear disease, with the exception of syphilis and Menière's disease. The vast majority of genetic, degenerative, systemic, and symptomatic inner ear lesions are completely beyond any rational treatment.

Once details of the hamster ear are known and the developmental timing of each structure is ascertained, a comparison to human ear development can be made The hamster can then be used as an experimental laboratory model to study the effects of viruses and drugs on ear development in the offspring of females treated during varying stages of gestation. The agents employed can be timed, their mode of damage studied (hemorrhage arrest of development, degeneration, etc.) and the severity of the lesion determined. Genetic factors in inbred strains can also be studied. Ten mutant genes have been described in the hamster eight of which are autosomal and two are totally X linked. All produce their most obvious effect on coat color although three have been found to be associated with morphological defects (Robinson, 1968).

The goals of these studies will be the investigation of any conceivable agents causing damage to the ear One application of these studies to humans is to reduce the incidence of hereditary prenatal, and postnatal degenerative types of deafness.

The following abbreviations are used in the illustrations

ac	anterior crus of stapes
al	*annular ligament*
b	body of incus
c	capitulum of stapes or cerebellum
ca	crista ampullaris
cd	cochlear duct
ch	cerebral hemisphere
ct	chorda tympani nerve
dm	double manubrium
ed	endolymphatic duct
ep	excavation for paraflocculus cerebelli
f	footplate of stapes
g	VIII nerve ganglia
hsc	horizontal semicircular canal
i	incus
iac	internal auditory canal
isj	incudo-stapedial joint
lp	lenticular process
lpl	long process of incus
ls	limbus spiralis
m	malleus
mc	*Meckel's cartilage*
mp	meatal plate
oc	organ of Corti
osb	osteoblasts
pc	paraflocculus cerebelli
rc	Reichert's cartilage
rm	Reissner's membrane
sa	stapedial artery
sc	semicircular canal
sl	spiral ligament
sm	stapedius muscle
sp	short process of incus
ssc	superior semicircular canal
st	scala tympani or stapedius tendon
stv	stria vascularis
sv	scala vestibuli
tc	tympanic cavity
ta	*tympanic annulus*
tm	tectorial membrane
tmm	tympanic membrane
ttm	*tensor tympani muscle*
VII	facial nerve
VIII	auditory nerve fibers

(*c*) Neonatal disorders

Most of the information we have about the pathology of kernicterus, anoxia, and hypoxia concerns the central nervous system, little is known about the morphological changes within the cochlea. It is possible that animal experiments could be performed in which hyperbilirubinemia deafness is produced and the pathological changes are identified.

(*d*) Congenital hereditary disorders

It is now well established that deafness is a common manifestation of many types of hereditary disorders, particularly the inborn errors of metabolism. Little is known about the morbid anatomy of progressive heredodegenerative sensorineural hypoacusis.

Much more detail is given in this chapter of the report from the National Institute of Neurological Diseases and Stroke, which covers all the areas of hearing disorders and needs for research in each specific area. Only those areas directly related to this study were included here. The most impressive message in this comprehensive report was felt to be what is the obvious, but sometimes overlooked fact, that information concerning pathological conditions, and their effects can only be interpreted and understood by appropriate comparison to data concerning the normal system.

THE PROBLEM OF EARLY INNER EAR DISEASE

Many attempts have been made in the past to solve the complexity of formulating a uniform classification of congenital hearing loss (Ormerod, 1960; Fraser 1964; Beal et al., 1967; Paparella & Winter 1968, and others). This study will refer to the term congenital in its strict definition—present at birth, and specifically to an anomaly of the middle and/or inner ear. The classification used will be as follows. (1) Hereditary; (2) Acquired *in utero*; (3) Acquired during birth.

This problem is elucidated further in more detail by Lindsay (1967); Kelemen (1965); Lindsay & Matz (1966); Cohn et al. (1968); and others. They find that the histopathological differentiation between genetically-determined and acquired types of congenital deafness often cannot be made. Altmann (1950) discussed a classification made by Steurer in 1926 which distinguishes between two types of inner ear deafness on the basis of the anatomic findings. One with regressive changes in the end-organ, the cochlear nerve, and its pathways, and the other with evident developmental anomalies only in the cochlea. He felt that the regressive changes develop either spontaneously leading to developmental anomalies and forming the anatomic basis of inherited deafness, or secondarily from extrinsic factors, the basis of acquired deafness. The anatomic picture must be differentiated from all known pictures of the different stages of spontaneous or secondary regressive changes of the inner ear.

The following classification has been rather universally accepted as providing a classical description of defects in fetal development or aplasia of the ear. This classification has been described by numerous authors for many years. For the sake of brevity the outline will be summarized from the presentations by Scheibe (1892); Altmann (1950, 1968); Ormerod (1960); Beal et al. (1967); Schuknecht (1967); Cohn et al. (1968); Gussen (1968); Paparella & Winter (1968). The order of presentation demonstrates the degree of agenesis in each type.

1. Total labyrinthine agenesis—Michel type. Complete lack of development of the internal ear first described in 1863 by Michel.

2. Cochleo-vestibular dysgenesis—Mondini-Alexander type: Development of only a single, flattened tube representing the basal coil of the cochlea with comparable underdevelopment of the vestibular system. Mondini gave the original description in 1791 of involvement of the cochlear bony structure as well as the length of the membranous cochlea. In 1907 Alexander added to this a description of malformation of the endolymphatic duct and sac.

3. Cochleo-vestibular malformation—Bing-

II Review of the Literature

A report prepared and published by the Subcommittee on Human Communication and its Disorders of the National Institute of Neurological Diseases and Stroke (Carhart, 1969) had the following objectives (1) to define the extent of the field of human communication and its disorders (2) to review the present status of research in human communication and its disorders, and (3) to outline unresolved problems and unmet needs with recommendations as to their solution Chapter 3 is entitled Research on Hearing and was prepared from information obtained by the Subcommittee with the aid of 68 consulting authorities in this area. Certain portions pertinent to the research of the literature of this thesis will be summarized

1 RESEARCH NEEDS

(a) Embryological development

Our understanding of the embryological development of the ear in relation to its function is at an elementary stage Among mammals only a few groups of animals—opossums, rats, mice and rabbits—have been examined sufficiently to show in a general way the course of structural differentiation and the development of effective hearing Many details are lacking and we need especially to understand the conditions that bring about the first stages of function Our knowledge of the development of the human ear is meagre and we know almost nothing about the functional relations Our uncertainty extends through the fetal period into the period of infancy and early childhood Here the embryological problems merge with problems of communication

Further work needs to be done on the embryology of the human ear and likewise on the ear of a number of animal species. This study should lead to a general comprehension of the embryology of the vertebrate ear Of particular interest is the series of developmental changes that mark the first emergence of auditory function and the refinements of performance up to the adult stage Of great importance is the study of hearing in infancy and its growth during the first days, months, and years of life Eisenberg (1970) has even more recently confirmed the findings of the Subcommittee in that there is a shocking paucity of normative information" on the development of hearing in early life This research should correlate anatomical and neural changes with changes in auditory function and general behavior and changes in the adequacy of communication

(b) Prenatal disorders

Additional new information on maternal viral infections and their effect on the fetus is needed. While the histopathological picture of aural embryopathy from maternal rubella is accumulating rapidly many other viruses most likely also affect the fetal hearing organ and may be responsible for many of the congenital hearing losses termed sporadic hereditary hearing loss.

Much more information is also needed about the influence of teratogenic drugs As the medical profession is continuously deluged by a growing array of new medications, advocated by the pharmaceutical houses for many ailments, there is continuing need for careful study of the effects of certain new drugs on the developing embryo

be classified into at least three types. The aplasias, characterized by varying degrees of incomplete development of the inner ear· the heredodegenerations, in which there is a progressive loss of hearing after the inner ear has developed normally· the chromosomal aberrations. Heredodegenerative deafness may occur alone or in combination with other abnormalities, in which case they are known as syndromes. These syndromes are often hereditary sometimes congenital sometimes abiotrophic, and sometimes mixed (Schuknecht, 1967).

Proctor & Proctor (1967) estimate that one third to one-half of all cases of congenital deafness are due to hereditary deafness. A more definite figure of one-half is given by Konigsmark (1969 *b*). It is estimated that in about 30–40% of all children born deaf the cause is unknown More children are surviving now because of better pre and post-natal care and because of general improvements in the care of the handicapped. For this reason the number of children with dual handicap (e.g. deafness with cerebral palsy deafness with low mental ability deafness with various congenital malformations) is increasing.

The abnormality of the inner ear in congenital deafness, which has been either directly damaged or incompletely developed, is irreversible The only hope, at present, in respect to direct treatment lies in preventive measures. Effort should be made to evolve preventive measures, for which more fundamental research is required. For example, it is important that the frequency and tendency of various known causes should be investigated (Livingstone, 1964).

Proctor & Proctor (1967) have listed 39 conditions with hereditary nerve deafness. In their survey they find that 90% of hereditary deafness is inherited as an autosomal recessive, and 10% as an autosomal dominant trait. A substantial number of patients with congenital deafness are homozygous for autosomal recessive mutant genes (Carter 1964). It was indicated in an analysis of a study of deafness by Chung et al. (1959), that about two-thirds of the patients were homozygous for autosomal recessive genes about one fifth were heterozygous for dominant mutant genes just a few were caused by sex-linked genes and about one-tenth were deaf from intrauterine and perinatal environmental causes. Most types of hereditary nerve deafness, both autosomal dominant, recessive, and from chromosomal aberrations, occur with associated defects (Schuknecht, 1967) However most cases of hereditary childhood deafness are not associated with an identified syndrome. Thyroid metabolism defect with or without goiter is the most commonly associated trait and appears to result from a variety of thyroid metabolism deficiencies of the recessive type. Brown (1967) states that disturbances of pigment metabolism are the only syndromes presently believed to be associated with dominant childhood deafness.

Analysis of the anatomic findings in man is aided by the fact that in certain animals, such as cats, dogs, guinea pigs and mice, inherited forms of deafness are known to exist (Altmann, 1950). There is a rapidly increasing knowledge of morphology of inherited deafness in mice and other rodents and in many respects the morphological findings are very similar or even identical with those in man, cats, dogs, or other animals except for the fact that in the majority of the strains of deaf rodents the hearing loss *is associated with* locomotor anomalies of a kind so *far never* observed in man, cats or dogs (Altmann, 1964 Deol, 1968) The locomotor disorders result from cerebellar degeneration, peripheral (vestibular), central, or both. In man the vestibular system is often normal in genetic deafness.

The golden hamster has been employed in several diverse areas of genetic investigation (Robinson, 1968) However there have been no reports of functional, embryological, or anatomical studies in relation to hereditary deafness. Magalhaes et al. (1962 *b*) studied heterochromia iridis in the hamster one of the

Siebenmann type The bony labyrinth is well formed, but the membranous part and particu larly the sense organ is poorly developed Siebenmann first described this type in 1902, with Bing adding associated abnormalities of the central nervous system in 1907

4 Cochleo-saccular hypoplasia—Scheibe type The vestibular portion of the inner ear is developed and functioning. Malformation is restricted to the membranous cochlea and saccule in a normal bony labyrinth Scheibe (1892) described this type, which is the type most frequently found (Deol, 1968)

5 Tympanic malformation—Siebenmann type Changes are found mainly in the middle ear such as typically seen in cases of Swiss cretins

The phylogenesis of the mammalian ear probably began with the lateral line system of fish which was needed to sense movement. The transition from water to land and air environ ment necessitated the development of a sound transformer and impedance matching mechanism The semicircular canals were the first to evolve from the lateral line system and the lagena was the second which later evolved into the cochlea (Taylor 1969)

Since the vestibular apparatus is hundreds of millions of years older than the later de veloped and more highly differentiated cochlear apparatus, it is well understood that for phylogenetic reasons the organs of recent development are far less resistant to disease than are the older organs (Goodhill, 1950)

Knowledge concerning congenital pathologi cal conditions of the ear can only be inter preted and understood by comparison of the normal development and structure of the ear Fraser (1959) terms this the embryologic approach in that knowledge of the abnormal must be based on knowledge of the normal in studying congenital malformations of the ear

3 HEREDITARY DEAFNESS

Hereditary deafness has been classified in many ways including the genetic classification of Martensson (1960) the clinically oriented classification of Kinney (1950) and the pathological classification of Ormerod (1960) Variations of these have been proposed by Goodhill (1950), and others. Konigsmark & McKusick (1966) feel that the simplest or ganization of types of hereditary deafness is based on associated anomalies, since the spe cific genetic defect in most types of hereditary loss results in abnormalities in other systems as well The following structures are involved in different types of familial hearing loss, ex ternal ears integumentary system visual system nervous system skeletal system urin ary system and endocrine system Thus, the types of hereditary deafness can be classified on the basis of associated defects caused by the same gene (Konigsmark 1969 *a*) These are known as syndromes and may be classified endodermal, mesodermal, and ectodermal, according to the combination of anomalies present (Schuknecht, 1967)

Fisch (1959) studied a hereditary syndrome with deafness the most significant clinical symptom but also involving the integumen tary system This study made possible a better understanding of the relation between body pigmentation and hearing. These two systems are both ectodermal in origin and thus closely linked developmentally and functionally and this link may explain some of the mechanisms involved which lead to anomalies appearing simultaneously in both systems. The laby rinth contains pigment for no known purpose, but its absence has been seen in hereditary deafness. Another striking coincidence is the simultaneous affliction of body hair and cochlear hair cells in certain syndromes, such as Waardenburg s syndrome (Waardenburg 1951) There is also a resemblance between the urinary system and inner ear because function of the stria vascularis is somewhat similar to kidney function (Ormerod, 1960)

Genetic factors, like infectious processes, vary greatly in the severity of their effect which is referred to as variability in specific ity Hereditary sensorineural hearing loss may

ard of medical care the possibility cannot, of course, be excluded that other as yet unsuspected, causes exist (Fraser 1964).

(c) Connatal syphilis

An excellent review of the literature on syphilis of the ear is presented by Goodhill (1939). He studied eight cases of congenital syphilis and found no specific variety of hearing loss that could be associated with luetic aural disease. Syphilis may be contracted during the passage of the infant through the birth canal, which would be intranatally acquired, or through hematogenous infection of the fetus through the placenta via the connecting maternal and fetal blood streams, a case of prenatal acquisition.

Karmody & Schuknecht (1966) have found *that there is considerable variation* both in time of onset and type of syphilitic hearing loss, as well as variation in the rapidity of its progression. The onset of early syphilitic deafness in childhood is usually very sudden, bilateral, symmetrical, profound, and not accompanied by marked vestibular symptoms. Hearing loss resulting from congenital lues may appear as early as the first decade or as late as the fifth (Patterson, 1968). Arnold & Ohsaki (1963) reiterated the well-known distinction of connatal syphilis of the ear into an early and late type. The nerve deafness of late connatal syphilis usually does not respond to treponemicidal therapy representing a hypersensitivity phenomenon.

(d) Neonate hyperbilirubinemia

Any condition causing hemolysis can cause a tissue accumulation of indirect bilirubin from hemoglobin breakdown, resulting in jaundice. Among these diseases are: Rh and AB0 incompatability cytomegalic inclusion disease, galactosemia, septicemia (sepsis), biliary atresia, and neonatal hepatitis. The primary cause of erythroblastosis foetalis is *considered to be* the presence of acquired Rh antibodies in the blood of an Rh negative woman (*false* blood transfusion, preceding pregnancy with Rh positive fetus) (Kelemen, 1956) While hyperbilirubinemia *is necessary for* the development of kernicterus, it cannot be considered the sole factor as hypoxia of varying degrees may be a necessary prerequisite for the development of bilirubin deposition in cranial regions (Goodhill, 1967).

In a study by Keaster et al. (1969) sensorineural hearing loss was found to occur with and without other central nervous system abnormalities. Findings from investigations by Matkin & Carhart (1968) *were contradictory* in determining whether congenital hearing losses were the result of anoxia (and thus of central origin), or peripheral, or a combination of both. Crabtree & Gerrard (1950) studied the brain stem and temporal bones of a neonate who died of erythroblastosis foetalis. They reported that both organs of Corti, auditory nerve fibers, and ganglion cells were normal. However the brain stem showed that *the cell bodies in both the ventral and dorsal* cochlear nuclei were absent. Damage of this type is thought to be caused by a direct toxic *effect of high bilirubin levels on the brain*, the deafness being due not to involvement of the peripheral auditory apparatus, but of the cochlear nuclei (Fraser 1964). There continues to be controversy over the site of the auditory lesion in Rh incompatibility (Sataloff & Vassallo, 1970). In his survey of congenital and acquired *cytomegalic inclusion infection*, Hanshaw (1966) reported at least one case of deafness in 13 infants afflicted by cytomegalic virus, after Hanshaw et al. (1965) had mentioned one case of congenital deafness among 9 virus-positive children.

(e) Toxoplasmosis

This has been well documented as an infection in pregnancy causing inner ear *anomalies* in fetuses (Kelemen, 1958 *b*). Toxoplasmosis is a ubiquitous infection so that elucidation of its role as a source of congenital deafness is *of first importance* (Kelemen, 1961).

features found in man with Waardenburg's syndrome (Waardenburg 1951) All the animals in which the trait was observed were belted or banded with white, which is a form of dominant white spotting in the hamster In both men and mice heterochromia iridis is sometimes associated with spotting genes

In hamsters, heterochromia iridis has not yet been observed in individuals with full or wild type pigmentation, or in animals in which the coat is entirely white The trait does not act as either a simple dominant or simple recessive and is as common in males as in females (Magalhaes et al 1962 *b* Robinson, 1968)

4 AURAL EMBRYOPATHIES (Human)

(*a*) Rubella

Hearing defects in the progeny of mothers who contracted rubella in the first trimester of pregnancy is a well studied phenomenon (Kelemen, 1966) The first description of a rubella epidemic was by Gregg (1941) which occurred in Australia in 1939–1941 He gave the first description of congenital cataracts associated with congenital heart disease in babies whose mothers had contracted rubella in the first trimester of pregnancy Swan et al. (1943) were the first to associate deaf mutism as a component of this entity in these rubella babies from the Australian epidemic A review of the congenital defects occurring in this epidemic were summarized by Swan (1944) Deafness associated with the rubella syndrome occurs frequently yet its pathogenesis is not established (Ingalls et al 1960 Dunnington, 1968) Intra uterine rubella infection may result in developmental retardation congenital malformations, and chronic inflammation in several visceral organs in addition to the classic triad of cataracts, deafness, and patent ductus arteriosus (Esterly and Oppenheimer 1969)

Hardy et al. (1969) recently observed that deafness and infant morbidity is not confined to maternal infection in the first trimester Fetal infection may take place at any time during pregnancy and long after delivery More recently Sever (1970) stated that rubella virus usually persists for 6 months after birth and has been reported by Plotkin (1970) to persist until 3 years of age in the lens of children with congenital cataracts. Sever (1970) feels that one possible viral mechanism of rubella infection through which organ development is disrupted is damage to blood vessels. A child who has a partial hearing loss at 5 or 6 years of age when the blood vessels damaged *in utero* by rubella virus hemorrhage under the increasing stress of the child's growth. Vascular vulnerability and insufficiency of the inner ear persists from the moment of first assault and manifests itself at any intra uterine or extra-uterine period (Kelemen, 1966)

A causal connection between maternal rubella deafness and the tendency toward hereditary hearing defects in the parents and their families was investigated by Anderson et al. (1970) in 26 out of 257 cases that were initially surveyed for maternal rubella hearing impairments. The results of this study indicated that a great number of the parents were carriers of genes for deafness and an overwhelmingly high incidence of hereditary deafness was present in the parents families. These authors concluded that genetic disposition for hearing impairment thus appears to be a prerequisite for maternal rubella deafness In man it would seem to be the first time that a connection has been found between a specific genetic defect in an organ and an exogenous agent—in this case rubella virus—acting on the same organ

(*b*) Measles

Lindsay & Hemenway (1954) reported that congenital deafness may be caused by measles during pregnancy but this is less common than maternal rubella Rubella is the only substantial known cause of prenatally acquired deafness today in countries with a high stand

after thalidomide exposure. Rosendal (1965) reported a case of a woman who had taken thalidomide between the 24th and 34th days ($3^1/_2-4^1/_2$ weeks) after conception. The infant died at 4 months of age. Histological study of the temporal bones revealed absence of the internal auditory meatus and of the acoustic and vestibular nerves bilaterally. A case of congenital labyrinthine aplasia in the offspring of a woman who had taken thalidomide 25 days (3 /₂ weeks) after conception was reported by Jørgensen et al. (1964). The infant died at 4 months of age and histological study was made of the temporal bones. Livingstone (1965) did a tomographic study of the labyrinth of children with thalidomide embryopathy and found arrested development of the inner ears with marked dilation of the semicircular canals.

(b) Lysergic acid diethylamide (LSD)

LSD has been suspected as having a causal relationship in newborn infants with deformities. Smart & Bateman (1968) made a thorough review of the literature on chromosomal damage and teratogenic effects caused by LSD in humans and animals. They came to the conclusion that the evidence for the teratogenic effect of LSD is very strong but somewhat ambiguous and incomplete.

Zellweger et al. (1967) reported a case of a pregnant woman who had taken LSD on the 25th day (3 / weeks) after her last menstrual period and three times between the 45th and 98th days (6 / – 14 weeks). Her husband had also taken LSD. His findings in the infant was a malformed right leg which included lack of fibula, anterior bowing of the shortened tibia, absence of the lateral rays of the foot, and dislocation of the hip. Chromatid breaks were found in the peripheral white blood cells of the infant and both parents. Hsu et al. (1970) reported a case where chromosomal analysis of the infant revealed trisomy 13 with a D/D translocation. The parents had used LSD prior to conception but not during pregnancy. The clinical findings suggested the combination of defects compatible with the Trisomy 13 syndrome.

Eighty to 85% of those using LSD manifest an unusually high incidence of chromosomal rearrangement (N.Y. Med. Soc. 1968). Similar chromosomal abnormalities have been found in some infants of women who took LSD during pregnancy. Thus, the danger of genetically induced damage in LSD users or their progeny is raised.

6. OTOTOXIC DRUGS

The ototoxicity of streptomycin, dihydrostreptomycin, neomycin, kanamycin, framycetin, gentamycin, salicylate, chloroquine, and quinine have been well documented in humans and animals, e.g. cat, monkey, guinea pig, rat (West, 1938; Glorig, 1951; Hawkins, 1959, 1967; Lindsay et al., 1960; Ward & Fernandez, 1961; Benitez et al., 1962; McGee & Olszewski, 1962; Engstrom & Kohonen, 1965; Kohonen, 1965; Finegold, 1966; Kreis, 1966; DeMoura & Hayden, 1968; Kerr & Schuknecht, 1968; Sataloff & Vassallo, 1970). There is agreement among these authors and others that these drugs have a predilection for the inner ear with resulting sensorineural deafness and/or vestibular disorders. There is also agreement that streptomycin produces a greater toxic effect on the vestibular system, while dihydrostreptomycin is more toxic to the auditory mechanism.

Since many drugs are known to be ototoxic, causing inner ear damage with resulting varied degrees of hearing loss, there seems to be little reason to doubt that congenital deafness may sometimes be caused by large doses of such drugs in pregnant women (Fraser, 1964). Robinson & Cambon (1964) reported on two children whose mothers had taken streptomycin during pregnancy for treatment of tuberculosis. In the first case the mother had been given a dose of 1 g twice weekly between the 6th and 14th weeks of pregnancy. Hearing loss was suspected in the infant at 6 months of age and at 4 years of age the child was

(f) Maternal toxic disease

A number of examples of maternal toxic conditions in the mother have demonstrated abnormal conditions in the ears of fetuses whose mothers had suffered, during the early months of pregnancy from some toxic or endocrine disease These embryopathies include diabetes, pseudohypoparathyroidism nephritis, poliomyelitis and viral pneumonia (Kelemen 1955 *a b* 1957 1958 *b* 1959 1960 Lindsay et al 1953 1954 Hinojosa 1958 Jørgensen 1961) According to Fraser (1964) congenital syphilis, toxoplasmosis and maternal rubella are the only aural embryopathies that are well substantiated

(g) Perinatal causes of deafness

The premature child is especially vulnerable to factors that may lead to deafness which occur in his struggle to adapt to the extra uterine environment (Fraser 1964) Deafness is more frequent in premature babies due to their fragility extra risks in labor and anoxia susceptibility all of which prediospose them to damage to the nerve cells of the cochlea (Harrison 1959) However Fraser (1964) states that anoxia and/or birth injury may also though less often, be responsible for deafness in normally mature children Fisch (1955) demonstrated that a high proportion of children with cerebral palsy have significant hearing losses Injuries within or near the temporal bone may cause hemorrhages in the inner ear (Kelemen 1965)

5 DRUG INDUCED MALFORMATIONS

(a) Thalidomide

Thalidomide was the first apparently non-toxic compound found to be teratogenic (McBride 1963) There can no longer be any doubt as to the causal relationship between the ingestion of thalidomide during pregnancy and congenital defects in the offspring involving not only the limbs but also the ears (Kleinsasser & Schlothane 1964) The results of a study by Lenz & Knapp (1962) suggested that the critical period when thalidomide ingestion may cause damage to the fetus is between the 37th and 50th day after the last menstruation In a few of their cases the time of conception was known and the critical period was from the 27th to 33rd day ($3\,^1/_2 - 4\,^1/_2$ weeks) of pregnancy Kittel & Saller (1964) studied a group of children that had been malformed by their mothers ingestion of thalidomide during pregnancy In this series of ear deformities, thalidomide had been taken around the fifth week of pregnancy suggesting that the teratogenic dose and the time of administration of thalidomide are related. The mode of action of thalidomide is not known but it is more important to know that a substance has a potential for damaging the fetus than to know the mode of action of such agents (Litchfield 1967)

The thalidomide embryopathy comprises a spectrum of widely different types (Smithells, 1965) In some children, the external ears are absent, and the facial and ocular nerves are paralyzed In others deafness due to a gross defect of the inner ear abducens paralysis, and preauricular appendages are the only signs (Lenz, 1966)

D Avignon & Barr (1964) studied 14 children whose mothers had taken thalidomide during the first two months of pregnancy Their findings suggested a complex syndrome with ear deformities, hearing loss, paralysis of the facial, abducens or other oculomotor nerves, paralysis of the soft palate, as well as other malformations of limbs, heart and other organs. It was notable that the syndrome of ear malformations, hearing loss, and cranial nerve paralysis was unaccompanied by limb deformities Kleinsasser & Schlothane (1964) studied in detail 70 children with thalidomide induced ear deformities. Their findings included, in addition to the well known forms of microtia various aplasias and hitherto unknown vestibular anomalies of the inner ear with deafness and absence of vestibular reactions Two cases were reported as the first observations of isolated ear malformations

er thalidomide exposure. Rosendal (1965) ported a case of a woman who had taken alidomide between the 24th and 34th days / –4 ¹/ weeks) after conception. The in- nt died at 4 months of age. Histological udy of the temporal bones revealed absence f the internal auditory meatus and of the coustic and vestibular nerves bilaterally. A ase of congenital labyrinthine aplasia in the ffspring of a woman who had taken tha- idomide 25 days (3 / weeks) after concep- ion was reported by Jørgensen et al. (1964). The infant died at 4 months of age and histological study was made of the temporal bones. Livingstone (1965) did a tomographic study of the labyrinth of children with tha- lidomide embryopathy and found arrested de- velopment of the inner ears with marked dila- tion of the semicircular canals.

(*b*) Lysergic acid diethylamide (LSD)

LSD has been suspected as having a causal relationship in newborn infants with de formities. Smart & Bateman (1968) made a thorough review of the literature on chromo- somal damage and teratogenic effects caused by LSD in humans and animals. They came to the conclusion that the evidence for the teratogenic effect of LSD is very strong but somewhat ambiguous and incomplete.

Zellweger et al. (1967) reported a case of a pregnant woman who had taken LSD on the 25th day (3 / weeks) after her last menstrual period and three times between the 45th and 98th days (6 / –14 weeks). Her husband had also taken LSD. His findings in the infant was malformed right leg which included lack of fibula, anterior bowing of the shortened tibia, absence of the lateral rays of the foot, and dislocation of the hip. Chromatid breaks were found in the peripheral white blood cells of the infant and both parents. Hsu et al. (1970) eported a case where chromosomal analysis of the infant revealed trisomy 13 with a D/D translocation. The parents had used LSD prior to conception but not during pregnancy. The clinical findings suggested the combination of defects compatible with the Trisomy 13 syn- drome.

Eighty to 85% of those using LSD manifest an unusually high incidence of chromosomal rearrangement (N.Y. Med. Soc., 1968). Similar chromosomal abnormalities have been found in some infants of women who took LSD dur ing pregnancy. Thus, the danger of genetically induced damage in LSD users or their progeny is raised.

6 OTOTOXIC DRUGS

The ototoxicity of streptomycin, dihydrostrep- tomycin, neomycin, kanamycin, framycetin, gentamycin, salicylate, chloroquine, and quin- ine have been well documented in humans and animals, e.g. cat, monkey, guinea pig, rat (West, 1938; Glorig, 1951; Hawkins, 1959, 1967; Lindsay et al., 1960; Ward & Fernandez, 1961; Benitez et al., 1962; McGee & Olszewski, 1962; Engstrom & Kohonen, 1965; Kohonen, 1965; Finegold, 1966; Kreis, 1966; DeMoura & Hayden, 1968; Kerr & Schuknecht, 1968; Sataloff & Vassallo, 1970). There is agreement among these authors and others that these drugs have a predilection for the inner ear with resulting sensorineural deafness and/or vestibular disorders. There is also agreement that streptomycin produces a greater toxic ef fect on the vestibular system, while dihydro- streptomycin is more toxic to the auditory mechanism.

Since many drugs are known to be ototoxic, causing inner ear damage with resulting varied degrees of hearing loss, there seems to be little reason to doubt that congenital deafness may sometimes be caused by large doses of such drugs in pregnant women (Fraser, 1964). Ro- binson & Cambon (1964) reported on two children whose mothers had taken strepto- mycin during pregnancy for treatment of tu- berculosis. In the first case the mother had been given a dose of 1 g twice weekly between the 6th and 14th weeks of pregnancy. Hearing loss was suspected in the infant at 6 months of age and at 4 years of age the child was

(f) Maternal toxic disease

A number of examples of maternal toxic conditions in the mother have demonstrated abnormal conditions in the ears of fetuses whose mothers had suffered, during the early months of pregnancy from some toxic or endocrine disease These embryopathies include diabetes, pseudohypoparathyroidism nephritis poliomyelitis, and viral pneumonia (Kelemen 1955 *a b* 1957 1958 *b* 1959 1960 Lindsay et al., 1953 1954 Hinojosa, 1958 Jørgensen 1961) According to Fraser (1964) congenital syphilis toxoplasmosis, and maternal rubella are the only aural embryopathies that are well substantiated

(g) Perinatal causes of deafness

The premature child is especially vulnerable to factors that may lead to deafness which occur in his struggle to adapt to the extra uterine environment (Fraser 1964) Deafness is more frequent in premature babies due to their fragility extra risks in labor and anoxia susceptibility all of which prediospose them to damage to the nerve cells of the cochlea (Harrison 1959) However Fraser (1964) states that anoxia and/or birth injury may also though less often be responsible for deafness in normally mature children. Fisch (1955) demonstrated that a high proportion of children with cerebral palsy have significant hearing losses. Injuries within or near the temporal bone may cause hemorrhages in the inner ear (Kelemen 1965)

5 DRUG INDUCED MALFORMATIONS

(a) Thalidomide

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20 weeks in the hamster but not in the other animals. The results for the newborn mouse were equivocal. In this study he also included adult albino rats, hedgehogs, and 3-week-old chicks, none displaying virus recovery. The chronic infection of rubella virus in the newborn ferret was also shown by Fabiyi, Gitnick & Sever (1967). In initial experiments by Oxford & Schild (1966), transplacental passage of rubella virus in the hamster could not be demonstrated. Further work in this area by Oxford & Sutton (1968) ga e the same findings. Preliminary studies by Oxford (personal communication) have suggested that even when rubella is inoculated directly into the amniotic sac in utero in rabbits and hamsters, no virus multiplication occurs. However Sever (1970) recently reported runted rabbits produced by infecting the does with rubella virus which persisted in the offspring tissues for many months. Mumps virus was injected intravenously in the pregnant hamster and also did not penetrate the placenta. It did not produce any histopathological lesions in maternal or fetal hamsters (Ferm & Kilham, 1963). It is believed that the viral macromolecules are too large to pass the placental barrier in these animals (Moya & Thorndike, 1962 Mitchell & Woodside, 1967).

(*b*) Thalidomide

In a study by Homburger et al. (1965) pregnant golden hamsters were fed a diet which contained 0.6% thalidomide. This resulted in grossly abnormal fetuses. The New Zealand white rabbit, rats, and mice were found to be sensitive to thalidomide in McBride's (1963) studies on its teratogenic action. No studies were found in the literature describing inner ear malformations from thalidomide in animals.

(*c*) LSD

Pregnant hamsters were injected with lysergic acid diethylamide (LSD), -bromo-D-lysergic acid diethylamide (BOL), and mescaline (MES) on the 8th day of gestation by Geber (1967) and sacrificed on the 12th day. His results showed that LSD MES, and BOL can induce a wide variety of malformations in the hamster embryo. Alexander et al. (1967) injected LSD in rats early in pregnancy which produced runts and increased fetal mortality. Injection late in pregnancy had no obvious effect on the offspring. In contrast to these findings, Warkany & Tokacs (1968) found no abnormalities in the offspring of rats injected with LSD on the 4th and 5th days of pregnancy even though the doses administered were as high as those used by humans. Auerbach & Rugowski (1967) injected mice with LSD on the 6th, 8th, and 9th days of pregnancy. Brain abnormalities were found in embryos when the mother had been injected in the earliest stage, but no gross observable effects occurred when the drug was administered later than the 7th day of gestation. The stage of pregnancy in mice found to be sensitive to embryonic malformations mediated by LSD is a stage equivalent to human pregnancy of 16–22 days.

8. DEVELOPMENT OF THE MIDDLE AND INNER EAR

(*a*) Human

The detailed description of the histogenesis of the developing inner and middle ear has been well presented by Streeter 1907 1918 1945 1948, 1951 Van der Stricht, 1918 Ingalls, 1920 Macklin, 1921 Bartelmez & Evans, 1926 Corner 1929 Bast, 1930 1932, 1936, 1938, 1940, 1942, 1944 1946- Anson, 1946, 1969- Anson et al., 1947 1948 *a* *b* 1955 Anson & Bast, 1946, 1949 1951 1958 Bast & Anson, 1950, 1952 Watzke & Bast, 1950 Richany et al., 1954 Bast et al., 1956 Heuser & Corner 1957 Borghesan, 1965 Van Alyea, 1965 The following is a brief summary of the developing middle and inner ear of the human.

Membranous labyrinth

The otic placode is believed to originate from both surface ectoderm, which forms the epi-

found to have a severe bilateral sensorineural hearing loss and total loss of vestibular function In the second case the mother received 1 g of streptomycin twice weekly during the last 4 months of pregnancy with the same findings in the child as in the first case

Five cases of reduced hearing in children of mothers who had received streptomycin or dihydrostreptomycin during pregnancy have been described since 1950 (Rasmussen 1969) This study showed that the danger of ototoxic damage to the fetus of a mother under treatment with these drugs during pregnancy is slight. However it confirmed earlier reports concerning the risk of hearing damage from treatment with large doses of either of these drugs. Bolletti & Croatto (1958) described a severe form of congenital deafness in a 5-year old girl resulting from streptomycin treatment of the mother during the last 3 months of pregnancy The findings of Conway & Birt (1965) suggested that further work is required to evaluate the risks to the fetus of streptomycin given during pregnancy

Chloroquine phosphate, which is chemically related to quinine may be an ototoxic drug in adults (Sataloff & Vassallo 1970) Matz & Naunton (1968) reported the case of a mother who had taken this drug for the treatment of systemic lupus erythematosus during the first trimestesr of her first, second, and third pregnancies The first child had bilateral profound sensorineural deafness, cerebellar dysfunction and marked bilateral vestibular paresis The second child was profoundly deaf and the third pregnancy resulted in miscarriage Three subsequent pregnancies without treatment with this drug produced normal children with normal hearing

7 EXPERIMENTAL TERATOLOGY (Animals)

The previous sections have described congenital and early acquired forms of hearing loss induced by prenatal and perinatal damage to the fetus and developing embryo of viral, infectious, metabolic birth traumatic, and pharmacologic origin Although the first trimester is considered to be that developmental stage when the human ear is most susceptible to teratogenic agents, literature was cited suggesting that aural embryopathies may also occur in the second and third trimesters of embryogenesis. There is sound evidence to indicate that the stage in development at which an agent is applied or becomes active is an important factor in determining the reaction of the embryo (Wilson 1957) Wilson (1959) feels that the basic action of all teratogens appears to produce either cell death or alteration in the rate of cell growth Susceptibility to teratogenic agents subsides to zero rapidly after the 8th week it increases for noxious agents because of the rapid growth and development of the placenta and the corresponding rapidly increasing ability of diffusible substances to reach the fetal circulation (Litchfield, 1967)

The golden hamster exhibits certain reproduction features which make it a desirable animal for research in teratology These include the ease of obtaining timed matings, large litters, and a mere 16 day gestation period (Ferm 1965 1967). This gestation period is shorter than in any other commonly available laboratory mammal (Magalhaes et al 1962 a) Hamsters are also readily obtained clean inexpensive and easy to maintain and handle The increasing value of the hamster as an excellent animal model for experimental pathology and clinical medicine and its potential value for experimental and comparative biology is discussed in detail by Homburger & Bajusz (1970)

(a) Viral studies

Oxford (1967) studied the persistence of rubella virus in newborn and adult hamsters, mice, ferrets, adult rabbits, and guinea pigs after intranasal inoculation of the virus His findings produced evidence for multiplication of rubella virus in the lungs of the rabbit hamster guinea pig, and ferret persisting for

Middle ear

The development of the middle ear begins at 4 weeks, at the same time the otic placode of the inner ear is deepening to form the otocyst. At about the 5th week a small pouch from the pharynx appears and comes into relation with the first branchial arch. This pouch forms the middle ear and is soon divided into a tubular portion, which becomes the Eustachian tube and a saccular one, which becomes the tympanum. The primitive Eustachian tube closes again during the 3rd month but reopens during the 4th. The head of the malleus and body of the incus are laid down in the mesenchyme of the first arch, the manubrium of the malleus and long process of the incus in mesenchyme of the second arch. The stapes develops from the 2nd arch about the 7th week. They are cartilaginous models at 9 weeks, and completely ossified at 26 weeks (malleus), 32 weeks (incus), and 35 weeks (stapes). In the 23rd week ossification starts in the medial and lateral parts of the tegmen tympani and is complete near the end of the fetal period

(*b*) Hamster

The literature on the development of the ear in the golden hamster is meagre, as confirmed by Kittel (1966) and Magalhaes (1968) in comprehensive bibliographies on the golden hamster inclusive of years 1931–1965 and comprising a combined total of 8 016 citations No articles dealing solely with ear development were found, with the exception of the ossification of the hamster ear by Van Arsdel & Hilleman (1951). Information on the ear was excerpted from articles describing the complete development of the hamster which therefore lacked details and completeness regarding the membranous labyrinth and middle ear development. This system was briefly described by Boyer (1948, 1953 1968), but it covered only the prenatal period. No articles were found dealing with the continuing postnatal growth of the ear Boyer (1953) stated that obvious gaps remained that should be filled in after further investigations, but be presented a general chronology that should be of value in providing landmarks for more detailed study

The gestation period of the hamster is 16 days with implantation of the blastocyst occurring on the 6th day post coitum (Venable, 1946 Ward, 1948) Once embryogenesis begins, it proceeds very rapidly from 8 to 10 days post coitum, then it slows somewhat after 10 days (Boyer 1948) The 13-day hamster has reached a stage in development equivalent to that of a 16-day rat, 24-day pig, and 60-day human (Magalhaes & Briggs, 1962).

The following is a presentation of the known information on the development of the middle and inner ear of the hamster In comparison with the summary previously presented on the human, it is clear that many areas need completion and definition The results of this study will present a comparative developmental timetable of the human and hamster ear

Day of gestation

8 Auditory pits appear and quickly begin approaching closure. They are in contact medially with wall of myelencephalon and anteriorly with VIII nerve primordium

9 Otic vesicle closed. Ganglion of VIII nerve becoming distinct and is plastered against otic vesicle which is no longer in contact with either brain or body wall and is laterally compressed with slender endolymphatic duct extending dorsally from its medial aspect.

10 Otocyst constricted into upper vestibular pouch and lower more compressed, cochlear pouch with slight demarcation of the latter into saccule and more slender incipient cochlea

11 Semicircular canals starting to differentiate. Auditory ossicles are present in precartilage mesenchymal condensations (beginning of otic capsule). VIII cranial

dermal layer of the skin and neural ectoderm which forms the neural structures (Pearson *et al* 1967) The placode can be recognized in a 3 week embryo as a diffuse thickening in the hindbrain region (Bartelmez, 1922) In the 4th week the placode rapidly invaginates forming the otic pit which deepens and fuses its edges to form a closed vesicle the otocyst The otocyst becomes separated from the nervous system about the 4th or 5th week of intra uterine life and nerve fibers can be seen leading from the ganglion to different parts of the vesicle The otocyst partly divides into two parts which develop as the cochlea and vestibule The cochlea consists only of the cochlear duct and at 6 weeks this is represented by a short curved tube, extending to a single complete turn at 7 weeks (Streeter 1918) The scalae vestibuli and tympani are developed out of the surrounding mesenchyme at this stage The full 2 1/2 spiral turns of the cochlea are reached at 9–10 weeks

Differentiation of the epithelial lining of the cochlear du t is first apparent at about 8 weeks It begins in the basal turn followed by the middle and later the apical turns At 12 weeks, there is a well marked group of cells in the basal turn which already bear a resemblance to the future organ of Corti and the tectorial membrane is even more definitely laid down At about the same time the neural elements of the VIII cranial nerve and the acoustic spiral ganglion have linked the central nervous system with the sensory epithelium in the developing sense organ

At 4 months the cochlea is almost in its adult form the organ of Corti being so advanced in the basal turn that the outer and inner hair cells can be identified and the inner spiral sulcus is formed The tectorial membrane is free in its mature form and position By the development of the scalae vestibuli and tympani, Reissner's membrane and the basilar membrane are formed. The sensory end organ is well developed in the middle turn but still represented by a simple ridge of sensory cells in the apical turn. At 6 months the inner ear and sensory end organ have reached their normal development throughout the cochlea the fetus is viable and the cochlea appears ready to function Several studies have indeed demonstrated fetal responses to sound after the 20th week of gestation (Eisenberg 1970)

Osseous labyrinth

When the membranous otic labyrinth is nearly completed, the otic capsule chondrifies and then ossifies from 14 different ossification centers forming the petrous part of the temporal bone The first center is formed on the outer part of the capsule over the basal turn of the cochlear duct at 15 weeks The last ossification center occurs in the region of the fissula ante fenestram and the oval window which starts to ossify in the 22nd to 23rd week

Ossification centers develop in relation to the internal auditory meatus, the semicircular canals, and the terminal branches of the VIII nerve Ossification of each part of the internal ear does not begin until that portion of the internal ear has reached its adult size (Bast 1930)

The human petrous bone thus remains in an embryonal state throughout life because it must be ossified at birth This fact is dictated by the very purpose of the cochlea to serve as an acoustic analyzer If the dimensions of such an acoustic instrument were to grow during childhood the acoustic sensitivity would change daily until maturity Such continuous changes in frequency response would preclude any learning of auditory patterns and thus of language and all human thought The price of this phylogenetic evolution is the susceptibility of the human petrous bone to a variety of hereditary bone diseases, including otosclerosis that leads to conductive or mixed hearing loss. Otosclerosis afflicts only the petrous portion (not the squama and tympanic ring) of the human temporal bone not occurring in any other human bones nor in animals.

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11 Semicircular canals starting to differentiate. Auditory ossicles are present in precartilage mesenchymal condensations (beginning of otic capsule). VIII cranial

nerve is present in general pattern of adult condition

12 Malleus is beginning to show definitive shape semicircular canals are complete cartilaginous otic capsule is formed middle ear cavity enlarging

13 Ossification is beginning in petrous bone auditory ossicles laid down in cartilage

14 Calcareous deposits forming beginning of utricular otolith and saccular otolith

15 First ossification center in prearticular portion of the malleus

16 BIRTH

Postnatal
day

2 (18) First ossification centers of cochlea and incus.

3 (19) Head and anterior process of malleus nearly completely ossified Ossification begins in the incus.

4 (20) Facial and internal auditory meatus outlined First indication of ossification around lateral semicircular canal

5 (21) First ossification center of stapes oval window incompletely outlined and stylomastoid foramen completely outlined.

8 (24) First ossification centers of superior and posterior semicircular canals, beginning ossification of tympanic bullae first ossification center in Eustachian foramen Ossification reaches base of manubrium of malleus.

10 (26) First ossification center of basic mastoid process.

11 (27) Ossification complete around semicircular canals manubrium of malleus ossified paraflocculus outlined petrous bone ossified except for a portion of the lateral wall in the region of the semicircular canals.

12 (28) Incus ossified

14 (30) Bullae ossified First otoconia appear on maculae

15 (33) Petrous bone ossified.

17 (33) Head of stapes ossified incudo-stapedial articular surfaces ossified with the articulation between the incus and stapes remaining free

21 (37) Tympanic annulus ossified

III Materials and Methods

Animals

Golden hamsters (*Mesocricetus auratus*) were used exclusively for all experiments, including the males used for breeding purposes. Ages ranged from embryos of 11 (Fig. 1) 13 (Fig. 2), and 15 days gestation (Fig. 3), post natal ages 1 3, 6, 9 12, 15 and 20 days, to three adults. Hamsters were obtained from a commercial breeder who furnished reliable data on ages of all postnatal animals and mating times and gestation periods for the animals used for the experiments on embryos.

One of the unique features of the golden hamster is that its introduction into the laboratory is precisely known. Professor I. Aharoni, while on a zoological expedition in Syria, was fortunate to capture several live specimens from the environs of Aleppo. An adult female and a litter of young were unearthed from a deep burrow Eight of the litter were eventually brought to the Hebrew University of Jerusalem Unfortunately as a result of various mishaps, offspring were obtained from only one male and two females. The capture occurred during April 1930, and by August of that year the first litter of hamsters were born in captivity The animals were made available to Dr S. Adler a colleague of Professor Aharoni, who sent breeding stock to various parts of the world, some of which arrived in the United States in 1938 (Fulton, 1968 Robinson, 1968). From such a humble beginning, all the golden hamsters now in use as laboratory animals in Europe and America are considered to be descendants of the three litter mates of 1930 (Adler 1948). It is from this stock that all present domesticated strains have been developed (Walker 1964).

Equipment

The laboratory used for this study is equipped with a paraffin dispenser oven, American Optical Company (AO) rotary microtome, and various microdissecting instruments. Microscopes available are a Bausch & Lomb Stereo-Zoom series B microscope, AO Spencer dual observation Cycloptic steroscopic microscope series 58, and an AO Spencer dual observation trinocular PhaseStar microscope series 10, model P4 with photographic attachment for polaroid and 35 mm cameras.

Gross anatomy experiments

An adult hamster weighing 130 g was chloroformed, decapitated, and skinned, including the eyeballs. Corrosion of the head was accomplished by boiling in water until the soft tissues were dissolved, leaving two halves of the mandible with one incisor tooth in each, two temporal bones, and a partially disassembled skull. These were reassembled, glued to the head of an ordinary straight pin and mounted on a piece of cork (Fig. 4).

Two adults, one weighing 127.5 g and the other 123 g, were anesthetized with 0.3 cc sodium pentobarbital diluted with 0.7 cc distilled H_2O Perfusion fixation of tissues was done following the method of Koenig et al. (1945). A midline incision was made to expose the heart and a cannula inserted into the ascending aorta through an incision made in the left ventricle. An incision was made in the right atrium to provide an exit for the fluids. The cannula was in communication with two reservoir bottles attached to tygon tubing and a glass Y tube One bottle contained saline which was used first to wash

out blood, and the other 10% formalin which was used immediately after the saline Both animals died during the perfusion procedure and were decapitated Each head was cut through the midline and preserved separately in 10% formalin The specimens from one animal were grossly dissected and studied under the StereoZoom microscope The right side of the other animal was preserved with the brain intact (Fig 5) and the left side after removal of the brain (Fig. 6)

Embryological experiments

The procedure for obtaining embryos was the same for all specimens Each was chloroformed an abdominal midline incision made and the entire uterus removed Each embryonic sac was separated by cutting the umbilical cord. The uterine membrane was sectioned for removal of the embryo This was done macroscopically for the 13- and 15-day embryos and microscopically with a dissecting microscope for the 11-day embryos All embryos were immediately placed in 10% formalin

A hamster in her second breeding was mated on May 22, 1968 and the above described procedure was performed on June 2 the 11th day of gestation, with removal of 11 embryos ranging in size from 5–7 mm C–R length A hamster in her third breeding was mated on May 17 1968 and on May 30 the 13th day nine embryos ranging in size from 13–16 mm C–R length were removed The first breeding of a hamster 8 weeks of age was on March 6 1968 and on March 21 the 15th day thirteen embryos ranging from 25–30 mm C–R length were removed

Postnatal experiments

The procedure for obtaining postnatal specimens was the same for all ages. The young animals were chloroformed and decapitated. In less than a minute all skin was completely removed and the apex of the cranium shaved with a razor for faster penetration of formalin The specimens were immediately placed in formalin with a change of solution every 24 hours for 2 days. The ages of these hamsters were 1 3 6 9 12, 15 and 20 days, the 1 3 6 and 9 day hamsters being obtained from the same litter

Histological procedure

Techniques used for all phases of specimen preparation and staining were those described by Lillie (1965) Altmann (1966) Humason (1967) and Luna (1968) in the Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology (1968) Specimens were fixed in 10% formalin for not less than 3 weeks. Decal (Scientific Products) was used for decalcification of all specimens, except the 11 and 13-day embryos for a period of time commensurate with the size of the specimen and amount of ossification Embryos and the 1 3 6 9 and 12 day postnatal specimens were embedded in paraffin and cut at 10 μ in the sagittal plane Fifteen

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Fig 1 Hamster embryo on the 11th day of gestation. Note the transparent area of the fourth ventricle with the cerebellum superiorly and the cervical flexure inferiorly The ear is forming lateral to the medulla oblongata. 12.

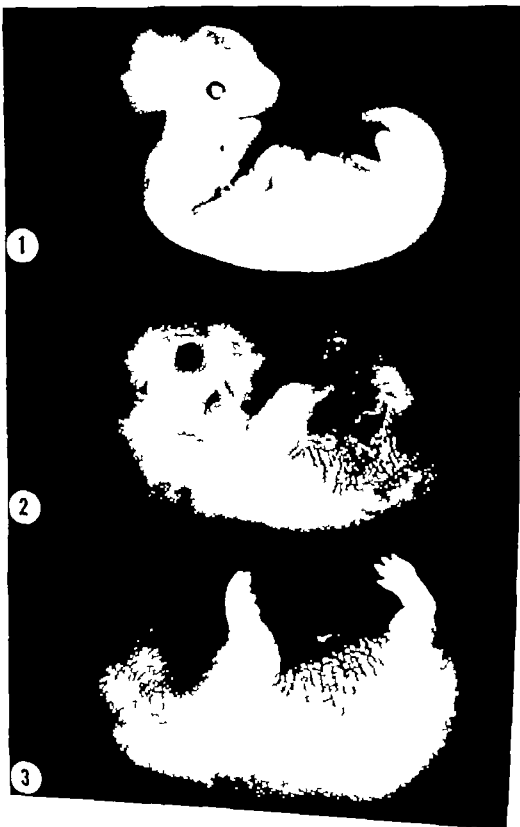
Fig 2 Hamster embryo on the 13th day of gestation. The ear is forming beneath the transparent skull. 6.

Fig 3 Hamster embryo on the 15th day of gestation. The pinna is developing on skin with hair follicles. Observe the astonishing rapidity of development of the embryo 6

Fig 4 Skull of adult hamster The right bulla shows the extreme thinness of the transparent bone. It is tilted laterally so that the medial upper surface is exposed, showing the excavation for the temporal lobe The left bulla is inclined medially showing part of the external auditory meatus. 1 7

Fig 5 Left half adult hamster head with the brain contained. The cerebellum (*arro*) and brainstem are easily identified 1 7

Fig 6 Left half adult hamster head after removal of brain tissue. The periotic capsu e is seen just lateral to the middle cerebral fossa. It contains a deep recess for the paraflocculus of the cerebellum (wh te area superiorly *arrow*) and the contents of the internal uditory meatus (small white area inferiorly *arrow*). 1.8.


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out blood, and the other 10% formalin which was used *immediately after* the saline. Both animals died during the perfusion procedure and were decapitated Each head was cut through the midline and preserved separately in 10% formalin The specimens from one animal were grossly dissected and studied under the StereoZoom microscope The right side of the other animal was preserved with the brain intact (Fig 5) and the left side after removal of the brain (Fig 6)

Embryological experiments

The procedure for obtaining embryos was the same for all specimens Each was chloroformed, an abdominal midline incision made, and the entire uterus removed. Each embryonic sac was separated by cutting the umbilical cord. The uterine membrane was sectioned for removal of the embryo This was done macroscopically for the 13- and 15-day embryos and microscopically with a dissecting microscope for the 11-day embryos All embryos were immediately placed in 10% formalin

A hamster in her second breeding was mated on May 22, 1968 and the above described procedure was performed on June 2, the 11th day of gestation with removal of 11 embryos ranging in size from 5–7 mm C–R length A hamster in her third breeding was mated on May 17 1968 and on May 30 the 13th day nine embryos ranging in size from 13–16 mm C–R length were removed. The first breeding of a hamster 8 weeks of age was on March 6 1968 and on March 21 the 15th day thirteen embryos ranging from 25–30 mm C–R length were removed

Postnatal experiments

The procedure for obtaining postnatal specimens was the same for all ages. The young animals were chloroformed and decapitated. In less than a minute all skin was completely removed and the apex of the cranium shaved with a razor for faster penetration of formalin The specimens were immediately placed in formalin with a change of solution every 24 hours for 2 days The ages of these hamsters were 1 3 6 9 12, 15 and 20 days, the 1 3 6 and 9 day hamsters being obtained from the same litter

Histological procedure

Techniques used for all phases of specimen preparation and staining were those described by Lillie (1965) Altmann (1966) Humason (1967) and Luna (1968) in the Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology (1968) Specimens were fixed in 10% formalin for not less than 3 weeks. Decal (Scientific Products) was used for decalcification of all specimens, except the 11 and 13-day embryos, for a period of time commensurate with the size of the specimen and amount of ossification Embryos and the 1 3 6 9 and 12 day postnatal specimens were embedded in paraffin and cut at 10 μ in the sagittal plane Fifteen

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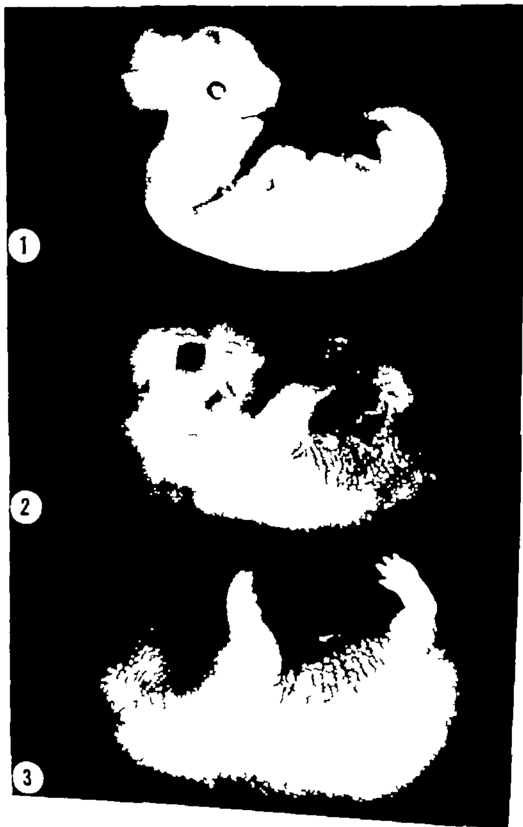
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and 20-day specimens were embedded in celloidin and cut at 20 μ. The 15-day specimen was cut in the horizontal plane and the 20-day in the sagittal plane.

Serial sections of embryos were made. Three 11-day embryos were processed, two stained with the routine method for Hematoxylin and Eosin Y and the other with the silver method of Gordon & Sweets (1936). Two 13-day embryos were processed, one stained with H & E and the other with a combined Periodic Acid Schiff (PAS)-Silver method (Wilson, Ashburn & Williams, 1968). A 15-day embryo was serially sectioned with the even numbered slides stained with the PAS-Silver method and the odd numbered with H & E.

Every 8th, 9th, and 10th section of the post natal specimens 1, 3, 6, and 9 days embedded in paraffin was stained routinely with H & E. Two 12-day postnatal specimens were histologically processed and embedded in paraffin. One specimen had every 10th section stained with the PAS-Silver method with serial sections of one ear being made with the other specimen and stained with H & E. Every 5th section of the 15- and 20-day specimens was affixed to a slide and stained with H & E.

Microscopic study

Slides were initially studied under low power with the StereoZoom microscope which had magnifications of 7× to 30× and the dual observation Cycloptic microscope with magnifications of 7×, 10×, 15×, 20×, 25×, 30× and 40×. Detailed study was done with the dual observation trinocular PhaseStar microscope with magnifications of 40×, 100×, 200×, 500× oil immersion, and 1 000× oil immersion. Dark phase contrast was also utilized with 100× and 200× magnifications. Dark contrast reveals the histologic detail dark against a lighter background.

Photomicrography was done with the polaroid camera attachment on the AO Spencer PhaseStar microscope. All available magnifications were used for various details, but the 40× and 100× were predominantly used for photography in order to show overall orientation of the development of the ear in each specimen.

IV Results

1 GROSS ANATOMY

This gross description was made with the dissecting microscope utilizing low and high powers *Approximative measurements* were estimated with a double-pointed compass and ruler with 0 5 mm divisions

The pinna is triangular in shape covered with hair on the outside and contains numerous cartilaginous convolutions near its insertion on the temporal bone Cerumen is seen as a shining coat over the inside cavity The pinna *is freely movable* in the living animal and shows a clear Preyer reflex to sound Several adult male and female hamsters weighing between 120 and 140 g were observed demonstrating this reflex, and subsequently for this anatomical description

Following removal of the pinna, the external auditory cartilaginous canal opening is oval in shape and measures 3.5 mm × 2.5 mm Numerous short hairs are seen in the skin lining the canal The bony canal is very short, round, and measures 2 mm in diameter at the isthmus The tympanic annulus forms an incomplete circle measuring 3 mm The superior incision of Rivini is seen It is closed by a well-developed Shrapnell's membrane The umbo is easily seen in the midportion of the tympanic membrane The tympanic membrane has a vertical position with slight inclination in a forward-medial direction From this angle results a fairly deep anterior recess of the external meatus

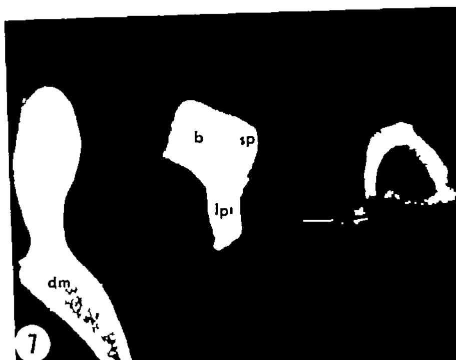
All tissue and muscle was removed from the large tympanic bulla which was observed to be oval shaped The lateral bony wall was gently removed until the medial aspect of the tympanic cavity was seen The bulla is formed by very thin bone that is transparent like oily paper and less than 0.25 mm thick The longer sagittal diameter of the bulla is 8 mm and the shorter transverse diameter is 4.5 mm The temporal bone is loosely connected to the base of the skull, separating readily by dissection as well as by boiling

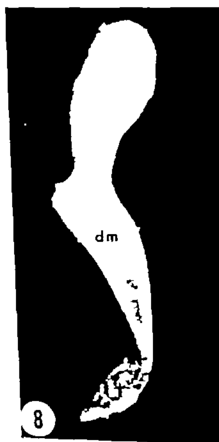
Approximately in the center of the medial tympanic wall is the promontory a round protruding mound The tympanic ostium of the Eustachian tube opens midway between the anterior pole of the bulla and the promontory Immediately in front of the promontory the apex of the cochlea can be seen The petrous bone is so thin at this point that a coil of the cochlea, presumably the apical turn, can be seen shining through it As expected the coiling runs clockwise in the left ear counterclockwise in the right Posterior to the promontory are seen the oval window superiorly and the round window inferiorly Both windows are less than 1 mm in their largest diameter and they are recessed within very deep niches The oval window niche contains the entire height of the stapes The two stapedial crura are in a line parallel to the long axis of the bulla i e the posterior crus is elevated from the horizontal line at an estimated angle of 15–20 degrees. The stapes is

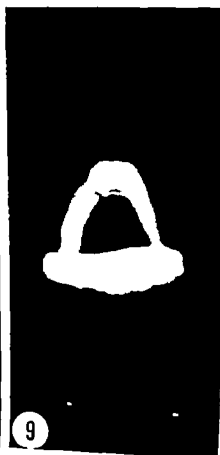
Fig 7 Malleus, incus and stapes of an adult hamster The irregular patch on the footplate of the stapes is a flake of modeling clay on which the ossicles were placed in order not to be blown by breathing (*arrow*). Photographed t 7 enlarged 3

Fig 8 High magnification of the malleus. Note the two bones of the manubrium and piece of tympanic membrane till attached. Photographed at 7 enlarged 6.5

Fig 9 High magnification of the stapes Photographed at 7 enlarged 5


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Fig 9 High magnification of the stapes. Photographed t 7 enlarged 5





Fig. 10. Middle and inner ear. 11th day of gestation. ×40

Fig. 11. Middle and inner ear. 13th day of gestation. ×60.

freely mobile moving inward and outward when one gently touches the capitulum with a needle

Through the obturator foramen of the stapes runs the typically persistent stapedial artery not visible to the unaided eye but almost filling the space. It crosses the stapes at a right angle and therefore courses from inferior–posterior in a direction upward and forward at an angle of 65–70 degrees from the horizontal line The bony canal of the stapedial artery which appears as a ridge over the promontory has an outside width of 1 mm In one specimen this canal is dehiscent just in front of the round window This artery runs in its own canal anterior and closely parallel to the facial nerve It enters the ventral aspect of the bulla, courses upward and forward and leaves the temporal bone near the internal auditory meatus, thus passing in the opposite direction to the efferent facial nerve

From the capitulum of the stapes extends the glistening tendon of the stapedius muscle in a posterior direction The muscle is fan shaped before it disappears inside the facial canal. No pyramidal eminence for the stapedius muscle is present The facial canal is bony but extremely thin its thickness being a fraction of the thin bone of the bulla The facial nerve is at least 1 mm in diameter and has the expected course beginning as the horizontal tympanic portion, then curving downward behind the oval and round windows, leaving the temporal bone at the stylomastoid foramen Posterior to the facial nerve is the *horizontal semicircular canal*, parallel to the curvature of the nerve and therefore inclined 30 degrees to the horizontal line

The stapes is less than 1 mm in height. The footplate is oval measuring less than 0.5 mm × 1 mm in diameter (Figs. 7 9) The incus is similar to the human form with its longest dimension being 1.5 mm It has a short process for its antral joint and a long process for the incudostapedial joint (Fig. 7) The malleus in the hamster varies from the human form in that its manubrium is composed of two parallel bones connected by a thin membrane, rather than of one solid rod as found in the human (Figs. 7 8) This double manubrium resembles the shape seen in rabbits. The entire malleus from capitulum to umbo measures 3 mm The lateral portion of the manubrium measures 2 mm and the medial portion slightly less than 2 mm The tensor tympani muscle courses in an open groove above the Eustachian tube Its tendon turns around a small cochleariform process, and inserts on a protuberance of the malleus neck.

Superior to the labyrinth is seen a large epilabyrinthine recess measuring approximately 2 mm in diameter It is lined by mucous membrane and no function can be deduced. The superior and medial walls are very thin, separating this air filled recess from the cranial cavity

The cochlea was opened and the scala tympani scala vestibuli cochlear duct Reissner's membrane and stria vascularis were seen with pigment being observed along the basilar membrane. The labyrinthine capsule was opened by scraping it with a scalpel and the semicircular ducts were seen in the expected positions. Finally the vestibule was opened, exposing the saccule anteriorly and the utricle posteriorly The acoustic nerve leaves through *the internal auditory meatus and enters the* brain stem

Study of the posterior aspect of the temporal bone revealed a large excavation posterior to the internal meatus. This recess measures 2 mm in diameter and contains the paraflocculus of the cerebellum a typical arrangement in rodents

Many other morphologic deviations from human anatomy were seen which may be worthy of special description but this was outside the scope of the present work

2 MICROSCOPIC ANATOMY

The following description will mention structures observed when making their *first* appearance Thereafter only those making de







Fig. 12. Middle ear and vestibular portion of the inner ear. 15th day of gestation. × 40.

Fig. 13. Manubrium of the malleus, chorda tympani nerve and tensor tympani muscle, 15th day of gestation. × 400.

Fig. 14. Osteoblast formation in preparation of ossification of the manubrium of the malleus and Meckel' cartilage, 15th day of gestation. × 200.

bone. The stapedius muscle has assumed its distinct fan-shaped form and is attached to the stapes. The tensor tympani muscle is composed of ery small bundle of myoblasts and has reached the level of attachment to the malleus (Fig. 13).

Inner ear (*Fig. 12*). The otic capsule is cartilaginous with the internal auditory canal

finite observable morphological changes will be discussed.

(a) Prenatal

11th day of gestation

Middle ear (*Fig 10*) Meckel s and Reichert s cartilages are still connected with the anlagen of the ossicles. The shape of the malleus, in cus and stapes is well defined in precartilage mesenchyme Mesenchymal condensations are forming the tympanic membrane in conjunc tion with the manubrium of the malleus an tero-lateral to which the ectodermal epi thelium has invaded the mesenchyme to form the meatal plate The stapedial artery is very small and lies next to the differentiating facial nerve and courses between the crura of the stapes. The chorda tympani nerve is repre sented by a small bundle of fibers which lie in the looser mesenchyme that fills the interval between the malleus and incus

Inner ear (*Fig 10*) Precartilage mesen chymal condensations are forming the otic capsule excepting one area that is forming the internal auditory meatus. One turn of the cochlea is present its capsule being formed of precartilage The epithelium of each cochlear duct has a thickened portion showing cellular differentiation which is destined to be the or gan of Corti Blood vessels, which are branches from the cochlear division of the internal auditory artery are numerous in the me senchyme which fills the area to be occupied later by the scalae vestibuli and tympani On one side toward the future modiolus some of these vessels become the blood supply of the stria vascularis Most of those in the rest of the mesenchyme appear to undergo degenera tion or are separated by such a great distance from each other as to become difficult to find The acoustic ramus of the VIII cranial nerve terminates in the proximity of the thickened portion of each cochlear duct The spiral ganglion cells are few in number and very immature The semicircular canals, saccule ampullae ductus reuniens, and endolymphatic duct are differentiating surrounded by pre cartilage mesenchyme.

13th day of gestation

Middle ear (*Fig 11*) The middle ear cavity has slightly enlarged and the meatal plate still persists The ossicles are distinctly outlined in dense precartilage mesenchyme differentiating to cartilage The pharyngeal pouch, which is continuous with the Eustachian tube is erod ing the mesenchyme which surrounds the ossicles. The manubrium of the malleus and body of the incus are still united with Meckel s cartilage The manubrium is observed as a narrow strip attached to the primordium of the tympanic membrane The tympanic mem brane has thickened stretched further and is attached to the developing tympanic annulus. The stapedial artery and facial nerve are de veloping as previously described.

Inner ear (*Fig 11*) The otic capsule is dif ferentiating from precartilage to cartilage The semicircular and cochlear ducts continue cellular differentiation The cells in the cochlear ducts begin secreting a substance that will form the tectorial membrane The mesenchyme on each side of the basal turn of the cochlear duct is beginning to break down to form the beginning of the perilymphatic system The canaliculus cochlearis is beginning to form from which the perilymphatic duct will be developed The VIII cranial nerve has divided into a superior and inferior portion Spiral ganglion cells have increased in number and maturity

15th day of gestation

Middle ear (*Fig 1?*) The ossicles are well formed in cartilage Osteoblasts are being formed from eroding cartilage in preparation of ossification between the manubrium of the malleus and Meckel s cartilage (Fig 14) Me senchyme is breaking down to form the synovial membrane between the malleus and incus. The tympanic membrane is attached to the tympanic annulus which is partially membrane







Fig. 12. Middle ear and vestibular portion of the inner ear 15th day of gestation. × 40.
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Inner ear (Fig. 12). The otic capsule is cartilaginous with the internal auditory canal

well hollowed out Four sections of the cochlear duct, presumably representing two turns of the cochlea (Fig 18) are differentiating with the organ of Corti demonstrating more advanced cellular differentiation (Fig 19) The tectorial membrane is beginning to show definitive shape Perilymphatic spaces around the semicircular canals are forming well with some connective tissue trabeculae Ampullae are undergoing cellular differentiation The crus commune joining the posterior and superior semicircular canals is evident. Maculae of the utricle and saccule show definite shape with cellular differentiation occurring The vestibule is clearly defined along with the oval window and ductus reuniens

(*b*) Postnatal

1 day

Middle ear (*Fig 15*) Meckel s cartilage is regressing The middle ear cavity contains a large amount of mesenchyme

Inner ear The basal turn of the cochlea is showing well defined structural development that is not present in the middle or apical turns (Fig 21) Two-and-one half turns are present (Fig 20) Reissner s membrane shows definite form with its attachment to the developing spiral limbus in the basal turn Sensory cells on the organ of Corti are beginning to rearrange in all turns of the cochlea but to a greater degree in the basal turn (Fig 21) to form the rods and tunnel of Corti The tectorial membrane still lies just above the cells and is attached to the spiral limbus in the basal turn and in the area in the middle and apical turns that will become the spiral limbus The scala vestibuli is not formed in the apical turn The amount of mesenchyme in the vestibular system seems to have reached maximum intensity (Fig 16) *Cristae ampullares* of the semicircular canals show increased cellular differentiation (Fig 17) The vas spiralis is showing further development. The expanded end of the endolymphatic duct is seen just lateral to the brain at *the lateral* recess of the fourth ventricle

3 days

Middle ear (*Fig 22*) Meckel s cartilage continues to regress Membrane bone occurs in the medial wall of the tympanic canal Ossification is progressing on the lateral side of the malleus and medial side of the incus with varying stages of osteoblast formation (Fig 24) The facial nerve canal is outlined in cartilage The tensor tympani muscle with its tendon is attached to the manubrium of the malleus The tendon of the tensor tympani muscle is chiefly formed by slightly condensed mesenchyme

Inner ear (*Fig 22*) The first ossification center in the otic capsule occurs The limbus spiralis is well formed in the basal turn of the cochlea and the cells on the organ of Corti are showing definite shape (Fig 25) The tectorial membrane has become increasingly free from the cells on the organ of Corti and shows recognizable form The ganglion cells of the VIII nerve have increased in number and maturity Cristae ampullares of the semicircular canals continue to show rapid neural development (Fig 23)

6 days

Middle ear (*Fig 26*) Those structures partially ossified are the body and short process of the incus, the capitulum of the malleus and the crura of the stapes. The Eustachian tube is seen surrounded by cartilage The tympanic membrane is laying down fibers with varying stages of development and the annulus tympanicus is well formed as membrane bone

Inner ear (*Fig 26*) The rods of Corti make their first discernible appearance as demonstrated by the rearrangement of hair cells with a very small open space in the area of the arcuate zone on the basilar membrane (Fig 28) The spiral ligament shows increased density The stria vascularis has not become very vascular The tectorial membrane is now freely extended from its attachment to the spiral limbus and has begun forming fibers The *semicircular canals have begun to ossify (Fig.* 27) Their perilymphatic spaces have a re







Fig. 15. Middle ear and vestibular portion of the inner ear 1 day postnatal. 20.
Fig. 16. Vestibular portion of the inner ear 1 day postnatal. 40.
Fig. 17. Crista ampullaris of the superior semicircular canal, 1 day postnatal. Photographed at 200, enlarged 3.

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3 days

Middle ear (Fig 22) Meckel's cartilage continues to regress. Membrane bone occurs in the medial wall of the tympanic canal. Ossification is progressing on the lateral side of the malleus and medial side of the incus with varying stages of osteoblast formation (Fig. 24) The facial nerve canal is outlined in cartilage The tensor tympani muscle with its tendon is attached to the manubrium of the malleus. The tendon of the tensor tympani muscle is chiefly formed by slightly condensed mesenchyme

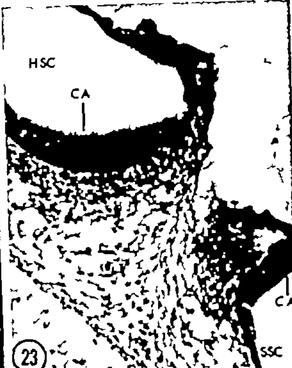
Inner ear (Fig 22) The first ossification center in the otic capsule occurs. The limbus spiralis is well formed in the basal turn of the cochlea and the cells on the organ of Corti are showing definite shape (Fig 25) The tectorial membrane has become increasingly free from the cells on the organ of Corti and shows recognizable form The ganglion cells of the VIII nerve have increased in number and maturity Cristae ampullares of the semicircular canals continue to show rapid neural development (Fig 23)

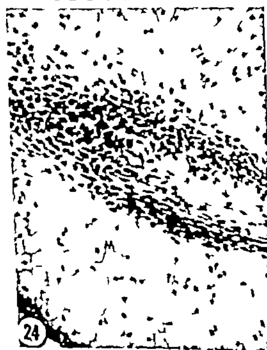
6 days

Middle ear (Fig 26) Those structures partially ossified are the body and short process of the incus, the capitulum of the malleus and the crura of the stapes The Eustachian tube is seen surrounded by cartilage. The tympanic membrane is laying down fibers with varying stages of development and the annulus tympanicus is well formed as membrane bone

Inner ear (Fig 26) The rods of Corti make their first discernible appearance as demonstrated by the rearrangement of hair cells with a very small open space in the area of the arcuate zone on the basilar membrane (Fig 28) The spiral ligament shows increased density The stria vascularis has not become very vascular The tectorial membrane is now freely extended from its attachment to the spiral limbus and has begun forming fibers. The semicircular canals have begun to ossify (Fig 27) Their perilymphatic spaces have a re







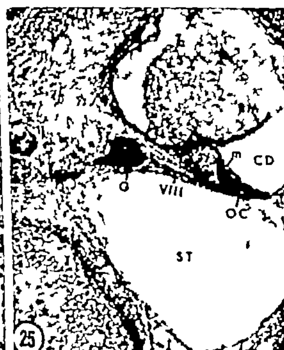


Fig. 22. Middle ear and vestibular portion of the inner ear 3 days postnatal, ×70.
Fig. 23. Cristae ampullares of the horizontal and superior semicircular canals, 3 days postnatal. ×200.
Fig. 24. Progressing ossification of the lateral side of the malleus and medial side of the incus, 3 days postnatal. ×200.
Fig. 25. Organ of Corti on the basal turn of the cochlea, 3 days postnatal. ×100.







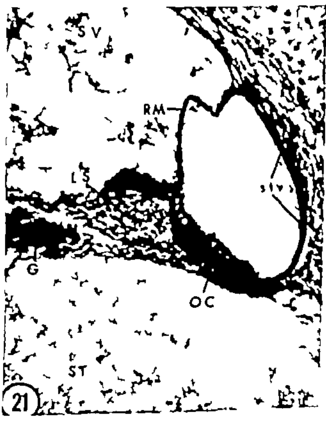


Fig 18 Otic capsule showing two turns of the cochlea, 15th day of gestation. 40

Fig 19 High magnification of the basal turn of the cochlea shown in the inset of Fig. 18. 400.

Fig 20 Otic capsule showing 2½ turns of the cochlea, 1 day postnatal 60.

Fig 21 High magnification of the basal turn of the cochlea shown in the inset of Fig. 20. 200.



Fig 29 Basal turn of the cochlea, 9 days postnatal. ×100.

one row of inner hair cells show a few hair like processes and immature cellular development. Claudius Hensen's, outer phalangeal cells of Deiter and inner phalangeal cells are observed in similar immaturity (Fig 29) The tectorial membrane has become increasingly fibrillar Maculae of the utricle and saccule show immature cellular development with only a few hair like processes apparent. Cupulae of the semicircular canals show few hair processes with immaturity of the hair cells of the cristae (Fig. 30)

12 days

Middle ear Ossification has advanced to most of the manubrium of the malleus, the long crus of the incus is completely ossified, and the footplate of the stapes has begun ossifying. The facial nerve is not well covered by its canal which is about half ossified. The canal of the chorda tympani nerve is almost completely surrounded by a thin layer of bone The tympanic membrane is still immature Mesenchyme is rapidly breaking down in the middle ear cavity The meatal plate has become separated from the tympanic membrane

Inner ear The petrous bone is approximately 75% ossified. The spiral ligament is well developed in the basal and middle turns of the cochlea (Fig. 31) but not in the apical turn The tectorial membrane is much further developed and fibrillar in the basal turn. The *space of Nuel is present* and the rods and tunnel of Corti further developed. The VIII nerve fibers are more numerous and well developed from the spiral ganglion cells along the basilar membrane and slightly beyond







Fig 6 Middle ear and vestibular portion of the inner ear 6 days postnatal 70.

Fig 27 High magnification of the cristae ampullares of the horizontal and superior semicircular canals shown in the inset of Fig. 26 The canals have begun to ossify (*arrow*) 75

Fig 28 Basal turn of the cochlea, 6 days postnatal 100

9 days

Middle ear Those structures ossified are the lateral process of the malleus and the long crus of the incus The facial canal is partially ossified.

Inner ear The rods of Corti are nearly formed Three rows of outer hair cells and

SV
RM
stv
tm
OC
viii
ST
31



Fig. 31. Basal turn of the cochlea, 1 days postnatal. ×40

Fig. 32. Crista ampullaris of the horizontal semicircular canal. Note the numerous nerve fibers, 1 day postnatal. ×475.



Fig 30 Crista ampullaris of the superior semicircular canal. Note the few hair processes and immature hair cells, 9 days postnatal. 475

their exit from the internal auditory meatus. The semicircular canals are almost completely ossified. Cristae ampullares of the semicircular canals are well developed with neural structures nearing completion (Fig 32)

15 days

Middle ear The malleus and incus are ossified except for the malleoincudal joint which remains cartilaginous. The manubrium of the malleus consists of two parallel bones connected by a thin membrane (Figs. 7 8) The bulla and petrous bone are ossified Bone of the bulla is observed to be extremely thin Mesenchyme continues to be abundant in the middle ear cavity The stapedial artery is seen in the obturator foramen of the stapes (Fig 34)

Inner ear A thin layer of bone surrounds all turns of the cochlea. The basilar membrane is developing well with increased cellular density and nerve fibers. Spiral ganglion cells continue to increase in size and number but are still immature All neural structures, the space of Nuel inner and outer tunnel pillars, and rods of Corti are clearly defined The spiral ligament shows increased density and appears stronger Reissner's membrane and stria vascularis are well developed. Pigment cells, first seen in the stria vascularis, are seen in the organ of Corti Fibers of the VIII nerve have increased in the internal auditory meatus and medulla. The semicircular canals and the parafloccculus recess are ossified Otoconia are seen in the maculae of the utricle and saccule with an increased number of hair cells observed The semicircular canals have well developed neural structures appearing ready to function (Fig 33)

20 days

Middle ear (Fig 35) Structures ossified are the facial canal tympanic annulus in its entire partial circle, the lateral surface of the stapes footplate and the head of the stapes and incudo-stapedial surfaces with a small amount



Fig. 35. Middle ear and ossified otic capsule. The paraflocculus cerebelli occupies a large portion of the capsule. 70 days postnatal. × 13.

Fig. 36. High magnification of the wall c crista ampullaris of the horizontal semicircu shown in Fig. 35. Note cupula and mature ment of numerous hair cells. × 200.





Fig 33 Crista ampullaris of the horizontal semicircular canal Note the increased umber of hair cells and nerve fibers, 15 day postnatal 400
Fig 34 Stapedial artery in the obturator foramen of the stapes, 15 days postnatal 40.

V Discussion

For an animal with such a short gestation period (16 days), implantation of the blastocyst is surprisingly slow not occurring until 6–7 days after fertilization, the same time as in the human with a gestation period of 270 days. Only 10 days remain for the fetal development *in utero* of the hamster As would be expected, hamsters are born in an embryonal state Newborn hamsters were observed to be able to stand and walk at 4 days of age although very limited with extreme incoordination. At 7 days they commence to eat small slivers of food pellets and have become much more active but are still fairly incoordinated. At 10 days of age they are able to reach the water bottle. Their eyes open at 16 days and coordination has improved. The hamsters are weaned at 22 days They continue to grow rapidly reaching maturity at 3? days, an age comparable to 13 years in the human The life span of the hamster is 2–3 years, a small fraction of the human life expectancy (70–75 years).

The anatomical relations of the hamster's middle and inner ear are relatively similar to the human A noteworthy difference is the persistence of the stapedial artery in the hamster which normally disappears during the third fetal month in human fetuses (House & Patterson, 1964) This rare abnormal persistence in humans has been associated with congenital sensorineural hearing loss, various other congenital abnormalities, skeletal abnormalities within the temporal bone, and tinnitus (Altmann, 1947 Kelemen, 1958 *a*, 1963). It presents a grave surgical hazard if encountered during operations on the ear The stapedial artery was consistently observed in the hamster at all ages described in this study

The manubrium of the hamster malleus is quite distinctive in its formation. Two small bones, one lateral and one medial, connected by a membrane form the malleus handle in contrast to one solid bone in the human. This arrangement is similar to that in the rabbit, but its significance is not known

The paraflocculus of the cerebellum is quite large and assumes an unusual position in the excavated center of the otic capsule. This space-saving development is typical for many rodents and apparently reflects their well-equilibrated agility

Developmental stages of the middle and inner ear of the hamster were compared to those of the human (Table I) by counting the hamster's embryonal stages in days and those of the human in weeks. This ratio of 1 7 reflects the astounding rapidity of ear development in the hamster In evaluating this comparison of the two developmental rates, a relationship of time in structural development could be deduced. The developmental times for both hamster and human are approximative for two reasons. The hamster produces a litter of 7–13 embryos, some of which may be more developed than others. In human embryos on the other hand, the precise developmental ages are but rarely known for certain because of the obvious limitations of available data.

Embryonal development begins at the same time in the hamster and human, 7 days after fertilization. The ear begins developing 1 day later in the hamster and 2 weeks later in the human. At this time the hamster ear develops rapidly in a period of acceleration to the 15th day of gestation which corresponds to a comparable developmental stage of the 15th gesta

of cartilage on the articulation between the incus and stapes. The tympanic membrane is well developed in an extremely large middle ear cavity that is relatively free of mesenchyme The stapedial artery remains persistent

Inner ear (*Fig 35*) The otic capsule is completely ossified The basilar membrane and the stria vascularis have thickened and matured with the tectorial membrane showing the same maturity The vas spiralis is seen just inferior to the basilar membrane Seen lateral to each turn of the cochlea is the cochlear ramus of the labyrinthine artery The development of the hair cells on the organ of Corti has advanced to a degree to suggest the possibility of response to sound. The bone around the semicircular canals, saccule, and utricle is very thin. The maculae of the saccule and utricle appear developed with numerous hair processes, nerve cells and fibers Cristae ampullares of the semicircular canals show mature development (Fig. 36). The perilymphatic spaces have become freer The paraflocculus is quite large occupying approximately 85% of the otic capsule (Fig 35) and appears mature with well differentiated Purkinje cells. The spiral ganglion cells and those of the ventral cochlear nucleus in the brainstem are more numerous and mature with many nerve fibers.

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Developmental stages of the middle and inner ear of the hamster were compared to those of the human (Table I) by counting the hamster's embryonal stages in days and those of the human in weeks. This ratio of 1 : 7 reflects the astounding rapidity of ear development in the hamster. In evaluating this comparison of the two developmental rates, a relationship of time in structural development could be deduced. The developmental times for both hamster and human are approximative for two reasons. The hamster produces a litter of 7–13 embryos, some of which may be more developed than others. In human embryos on the other hand, the precise developmental ages are but rarely known for certain because of the obvious limitations of available data.

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Table I *Development of the middle and inner ear*

Hamster days	Structure	Human, weeks
6–7	Blastocyst attaches to uterine mucosa	1
8	Otic placode middle ear begins development	3
9	Otocyst VIII nerve ganglion cells developing	4
10	Stapedial artery VIII nerve and chorda tympani begin developing VIII nerve fibers from otocyst precartilage mesenchyme forming otic capsule	5
11	3 semicircular ducts forming ossicles precartilage	6
	Saccule, utricle, maculae, crus commune, ductus reuniens, *scala tympani and cristae ampullares begin developing*	7
	One turn of cochlea precartilage otic capsule, organ of Corti begins developing; tympanic membrane, endolymphatic duct stapedius muscle begin developing	8
13	2½ turns of cochlea (Human) ossicles cartilaginous otic capsule cartilage scala vestibuli beginning of perilymphatic system, tympanic annulus and tectorial membrane begin developing	9
14	Otolithic membrane of maculae and cupulae of cristae, tensor tympani muscle	11
15	First ossification center of malleus tympanic annulus membrane bone; stapedial artery disappears in human 2 turns of cochlea (Hamster)	15
Birth–16		
1 (17)	2½ turns of cochlea (Hamster) Meckel's cartilage regressing	15
(18)	First ossification center of cochlea and incus	15
3 (19)	Malleus and incus ossifying; tympanic membrane and tympanic annulus well formed	16
6 (22)	Semicircular canals, stapes, and facial canal begin ossifying	18
9 (25)	Cells on the organ of Corti are recognizable and are differentiating to form the rods and tunnel of Corti stria vascularis is well developed	20
12 (28)	Meatal plate hollowed out	21
15 (31)	Malleus ossified semicircular canals ossified incus ossified (Human incus: 32 w.)	26
20 (36)	Membranous and osseous labyrinth developed middle ear developed Stapes ossified	28 35 36 *Birth*

tional week in the human (1 day 1 week ratio) The hamster is born on the 16th day in an embryonal state similar to the 15th gestational week in the human Development of the ear proceeds in a period of deceleration with the ear being essentially completed at 20 *days of age (5 weeks from fertilization)* corresponding with the 28th gestational week in the human

The development of the ear during the 11th day of gestation in the hamster shows remarkable rapidity Structures that take only 1 day to begin developing take 3 weeks in the human for similar structural development Ossification of the incus and stapes takes somewhat longer in the human as compared in time to the hamster resulting in a return to the 1 1 ratio observed After the acceleration period, this ratio is reached at the 15th day of gestation in the hamster and 15th week of gestation in the human Deceleration ensues with the final development taking 20 days in the hamster as compared to the initial acceleration period of 10 days in the hamster and 21 weeks in the human as compared to 15 weeks of initial accelerated development time At the time the stapes becomes ossified (36 days from fertilization in the hamster and 35 weeks in the human) a 1 1 ratio has once again emerged

Table II. *Development of the organ of Corti*

Hamster, days	Structure	Human, weeks
8	Otic placode	3
9	Otocyst	4
11	Cochlear duct	5
	One turn of cochlea, organ of Corti begins developing—inner ridge cells develop spiral limbus, outer ridge becomes organ of Corti	8
13	Tectorial membrane begins developing	9
15	2 turns of cochlea	9
Birth–16		
1 (17)	2½ turns of cochlea, Reissner's membrane	9
9 (25)	Differentiation of cells on organ of Corti occurring first in basal turn progressing to apical turn—outer ridge differentiates into inner and outer hair cells, Hensen's, pillar and supporting cells	20
20 (36)	Organ of Corti formed	23

The development of the organ of Corti (Table II) also shows interesting time relationships. The development of the sensory cells was observed to be faster in the hamster occurring in 13 days whereas the human equivalent in development occurs in a period of 18 weeks. However the 2 $^1/_2$ turns of the cochlea are developed sooner in the human than the hamster in a time relationship. By the 9th week the human has attained the full turns in a time period of only 1 week corresponding to a development period of 6 days in the hamster to attain 2 $^1/_2$ turns.

The hamster reaches maturity at 32 days after birth, compared to the human at around 680–740 weeks reflecting a marked delay. The life span of the hamster is generally 2–3 years corresponding to 70 years in the human, not a ratio of 7 times but 25–30 times. All stages of the hamster's life, embryogenesis, birth, maturation, and life span thus reflect the short time available for its life

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12 (28)	Meatal plate hollowed out	21
15 (31)	*Malleus ossified, semicircular canals ossified incus ossified (Human incus: 32 w.)*	6
20 (36)	Membranous and osseous labyrinth developed middle ear developed Stapes ossified	28 *35* *36 Birth*

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mentelles Versuchstier für das Studium der angeborenen Taubheit.

Diese Untersuchung zeigte, daß die Ohrentwicklung des Hamsters außerordentlich rasch vor sich geht, indem sie 20 Tage nach Geburt im Wesentlichen abgeschlossen ist. Eine Zeitspanne beschleunigten Wachstums zeigte sich zwischen den 8. und 15. Tagen der Gestation, welches der 3. bis 15. Woche der menschlichen Fötalzeit entspricht. Eben diese Zeitspanne der embryonalen Entwicklung ist dafür bekannt, daß sie für teratogene Schädigung besonders empfindlich ist, wozu auch Ohrschäden gehören. Eine weitere Periode von verlangsamter Entwicklung des Ohres ergab sich für die Gestationstage 15–20 beim Hamster im Vergleich zu einer gleichartigen Verlangsamung während der Schwangerschaftswochen 15–28 beim Menschen.

Der Hamster wird im embryonalen Zustand 16 Tage nach Empfängnis geboren, er erreicht sexuelle Reife 32 Tage nach Geburt, und hat eine Lebenserwartung von 2–3 Jahren. Das Dasein des Hamsters ist im Vergleiche zum Menschen noch mehr verkürzt als seine embryonale Entwicklung, welches im Laboratorium große Zeitersparnis bedeuten kann. Aus verschiedenen solchen Gründen erfreut sich der Hamster zunehmender Beliebtheit als ein gut brauchbares und praktisches Laboratoriumstier.

Résumé

Plusieurs agences professionelles ont rapporté le grand nombre de personnes qui souffrent à ce des pertes de l'ouïe de different degré. Ces agences soulignent aussi le grand besoin de recherches intensifiées en plusieurs champs afin de mieux clarifier la condition incapacitante que represente la sourdité. Une de cettes régions est le développement des models d'animal pour étudier les effets des substances tératogéniques qui peuvent insulter l'oreille pendant son développement de l'embryon. La pathologie de certaines autres formes de maladie de l'oreille qui attaquent après la naissance doit également être mieux clarifiée.

Ici est décrit pour la première fois le développement de l'oreille moyenne et intérieure chez le hamster. La séquence temporale des structures a été comparée avec celle chez l'homme. Une relation de 1 : 7 jours chez le hamster : vers semaines chez l'homme, est apparu pour comparaison. En précisant les details structurelles de l'oreille du hamster on a donc rencontré un animal expérimental de laboratoire pour l'étude de la surdité congénitale.

Les résultats de cette exploration ont montré que le développement de l'oreille chez le hamster procède très rapidement, étant essentiellement complété vingt jours après naissance. Une période de développement accéléré a été trouvée pendant les jours 8–15 de gestation, comparable aux semaines 3–15 de la gestation humaine. Pendant cette même période, le développement foetal est *très receptif* envers des substances tératogéniques *qui* peuvent causer des anomalies de l'oreille. Une période de décélération de *développement aural* s'est montrée pendant les jours 15–20 de gestation chez le hamster comparée avec les semaines 15–28 de gestation humaine.

Le hamster est né dans un état embryonnaire 16 jours après fertilisation. Il est mature et capable du reproduction à l'âge de 32 jours et il a une expectation de vie de 2–3 ans environ. Ainsi, son existence est même plus raccourci que sa vie embryonale, un fait très économique dans le laboratoire. Pour ces raisons variées, le hamster est devenu très populaire comme animal util et pratique dans le laboratoire.

Summary

An appallingly large number of humans suffering from varying degrees of hearing impairment have been reported by many agencies These groups stress the dire need for research in many areas to shed light on this disabling condition One of these areas is the development of animal models to study the effects of teratogenic agents damaging the ear during embryogenesis The pathology of other known types of ear disease after birth is also greatly needed

The development of the middle and inner ear of the golden hamster has been described for the first time The developmental timing of structures has been compared to the human A 1 7 time ratio days in the hamster to weeks in the human, was used for this comparison By establishing the structural details of the hamster ear an experimental laboratory animal for the study of congenital deafness has been developed

The results of this study have shown that the hamster's development of the ear proceeds extremely rapidly being essentially completed at 20 days after birth A period of accelerated development occurred from 8–15 days of gestation comparable to the 3rd to 15th weeks of human development This has been reported to be the period of fetal development most susceptible to teratogenic agents that cause ear anomalies. A period of deceleration in ear development occurred from the 15th day of gestation in the hamster and 15th week of gestation in the human until approximately 20 days of age in the hamster and 28 weeks gestation in the human.

The hamster is born in an embryonal state at 16 days after fertilization reaches maturity at 32 days of age and has a life span of 2–3 years. The hamster is enjoying increased popularity as a useful and practical laboratory animal

Zusammenfassung

Eine erhebliche Zahl von Menschen leiden an verschiedenen Graden von Hörverlust, wie von vielen offiziellen Körperschaften berichtet Diese Verwaltungsstellen betonen auch das ernste Verlangen nach mehr Forschung, um diese schweren Körperbehinderungen besser zu verstehen Eines dieser Forschungsgebiete betrifft die Entwicklung von Tiermodellen zum Studium der Auswirkungen von teratogenen Stoffen welche das Ohr während der Embryogenese schädigen können. Auch sollte die Pathologie anderer bekannter Arten von Ohrenkrankheit nach der Geburt besser erforscht werden

Hier ist zum ersten Male die Entwicklung des mittleren und inneren Ohres des Goldhamsters beschrieben Die zeitliche Entwicklungsfolge der Ohrstruktur des Hamsters wurde mit der menschlichen Ohrentwicklung verglichen Dabei ergab sich ein Zeitverhältnis von 1 7 was der Hamster in Tagen entwickelt braucht beim Menschen ebenso viele Wochen Aus diesem Nachweis der Entwicklung des Hamsterohres ergab sich ein experi

mentelles Versuchstier für das Studium der angeborenen Taubheit.

Diese Untersuchung zeigte, daß die Ohrentwicklung des Hamsters außerordentlich rasch vor sich geht, indem sie 20 Tage nach Geburt im Wesentlichen abgeschlossen ist. Eine Zeitspanne beschleunigten Wachstums zeigte sich zwischen den 8 und 15 Tagen der Gestation, welches der 3 bis 15 Woche der menschlichen Fötalzeit entspricht. Eben diese Zeitspanne der embryonalen Entwicklung ist dafür bekannt, daß sie für teratogene Schädigung besonders empfindlich ist, wozu auch Ohrschäden gehören. Eine weitere Periode von verlangsamter Entwicklung des Ohres ergab sich für die Gestationstage 15–20 beim Hamster im Vergleich zu einer gleichartigen Verlangsamung während der Schwangerschaftswochen 15–28 beim Menschen.

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Résumé

Plusieurs agences professionelles ont rapporté le grand nombre de personnes qui souffrent avec des pertes de l'ouïe de different degré. Ces agences soulignent aussi le grand besoin de recherches intensifiées en plusieurs champs afin de mieux clarifier la condition incapacitante que represente la sourdité. Une de cettes régions est le développement des models d'animal pour *étudier* les *effets* des substances tératogéniques qui peuvent insulter l'oreille pendant son développement de l'embryon. La pathologie de certaines autres formes de maladie de l'oreille qui attaquent après la naissance doit également être mieux clarifiée.

Ici est décrit pour la première fois le développement de l'oreille moyenne et intérieure chez le hamster. La séquence temporale des structures a été comparée avec celle chez l'homme. *Une relation de* 1 : 7 *jours* chez *le* hamster vers semaines chez l'homme, est apparu pour comparaison. En précisant les détails structurelles de l'oreille du hamster on a donc rencontré un animal expérimental de laboratoire *pour l'étude* de la surdité congénitale.

Les résultats de cette exploration ont montré *que le développement de l'oreille* chez le hamster procède très rapidement, étant essentiellement complété vingt jours après naissance. Une période de développement accéléré a *été* trouvée pendant les jours 8–15 de gestation, comparable aux semaines 3–15 de la gestation humaine. Pendant cette même période le développement *foetal* est très receptif envers des substances tératogéniques qui peuvent causer des anomalies de l'oreille. Une période de décélération de développement aural s'est montrée pendant les jours 15–20 de gestation chez le hamster comparée avec les semaines 15–28 de gestation humaine.

Le hamster est né dans un état embryonnaire 16 jours après fertilisation. Il est mature et capable du reproduction à l'âge de 32 jours; et il a une expectation de vie de 2–3 ans environs. Ainsi, son existence est même plus raccourci *que sa vie embryonale*, un fait très économique dans le laboratoire. Pour ces raisons variées, le hamster est devenu très populaire comme animal util et pratique dans le laboratoire.

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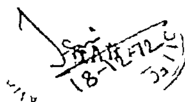
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SUPPLEMENT 297

Cochlear Physiology and Hair Cell Population in a Strain of the Waltzing Guinea Pig

BY

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Introduction

Our knowlege of the inner ear morphology in sensorineural hearing impairments in man is still largely deficient. In animals, the morphologic and electrophysiologic changes associated with cochlear lesions have been thoroughly investigated, but less so the course and the characteristic of the resulting hearing impairments. Furthermore one of the more extensively studied animals, with the most readily accessible cochlea, the guinea pig has stubbornly withheld its cooperation in hearing threshold determinations—until, in 1965 Anderson and Wedenberg described a new method for performing hearing threshold determinations in this animal. In model experiments they found that in hearing tests the Preyer reflex behaves similarly to the stapedius reflex in man.

An increasing number of sensorineural hearing impairments in man have been ascribed to genetic defects. It has even been possible to detect and define inherited subclinical stigmata in otherwise clinically normal-hearing carriers (Anderson & Wedenberg, 1968). In animals there are a large number of mutants with genetic deafness, and these, especially in rodents, often display behaviour al disorders which are conceivably due to concomitant vestibular dysfunction.

A strain of the waltzing guinea pig, originating from six waltzers received from the National Institutes of Health in 1961 has been bred and studied at the King Gustav V Research Institute. Genetic analysis has disclosed a dominant mode of inheritance with a recessive lethal effect (Ernstson, 1970) The vestibular function of the waltzers was severely impaired already at birth, and in the adult animals it progressively deteriorated to total loss of vestibular responses (Ernstson, 1971 a). The vestibular end-organs—the cristae and the maculae—showed progressive degeneration of the sensory cells, with a specific proteinoid rod shaped inclusion body in the type I hair cells. The nerve endings and the ganglion of Scarpa were normal in appearance (Ernstson 1971 a Ernstson et al., 1969 1970).

Progressive degeneration of the cochlear neuroepithelium, obvious already in the immediate post-natal period, eventually rendered the organ of Corti practically devoid of hair cells within 6–8 weeks of birth (Ernstson 1971). Degeneration of the supporting cells followed, and the spiral ganglion later displayed increasing depopulation of neurons. Other intra-cochlear structures were essentially normal in appearance. A special study of the cochlear vascular anatomy revealed no anomalies, even in the old waltzers (Axelsson & Ernstson, 1972, in press).

The aim of the present study was to elucidate the course and character of the hearing impairment and the cochlear hair-cell depopulation pattern in our strain of the waltzing guinea pig.

Material

This investigation was performed on 95 waltzing guinea pigs ranging in age from a few hours to about two months. A control group of 120 normal guinea pigs with a similar range of age was used.

Methods

The hearing measurements were carried out in a sound-proof chamber that effectively ex cluded environmental noise. Hearing thresh old determinations were performed by the method of Anderson and Wedenberg (1965) this is based on a peculiar characteristic of the guinea pig whereby on exposure to cold, it responds with periodic shivering of extreme regularity Previously conditioned animals re-act to a perceived tone stimulus with aboli tion of the shivering or a decreased shiver rate For complete details of technique and equipment the reader is referred to the ori-ginal paper Attenuator steps of 10 dB were chosen for the hearing threshold determina tions the following test frequencies were used 125 250 and 500 Hz; 1 1 1/2, 2, 3 4 8 and 12 kHz.

For Preyer reflex measurements carried out concomitantly with audiometry a spotlight was focused on the pinnae and the reflex ob-served through a window in the sound proof chamber For all the reflex determinations the test frequencies used were 0 5 1 2 3 and 4 kHz, with attenuator steps of 5 dB Preyer reflex measurements in the immediate post natal period were performed in the animal's compartment with a compact mobile version of the apparatus described by Wedenberg (1963)

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level was zero dB. The median threshold of hearing corresponded remarkably closely to that found in man for a free-field presentation (ISO R 226). For use as a reference in further audiometry modal values were chosen (Table I), and in the following audiograms these values constitute the zero level.

The Preyer reflex thresholds were determined concomitantly in the 30 normal guinea pigs, the range and the median values are shown in Fig. 1. In the following audiograms the normal range is given (shaded area).

By very early conditioning of newborn guinea pigs it was possible to perform reliable hearing threshold determinations during the first week of life—earliest on the third day. Four normal guinea pigs were subjected to early audiometry and re-examined repeatedly. Not until about 10 days post partum were normal hearing threshold levels attained. An example is shown in Fig. 2. The Preyer reflex levels were within the normal range throughout the post-natal period.

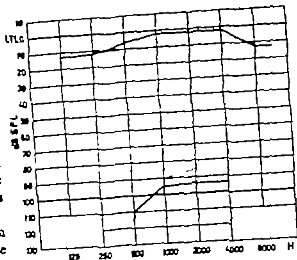


Fig. 1. Normal threshold of hearing for 30 adult guinea pigs (above), and normal Preyer reflex threshold (below). SPL values. Shaded area, total range; continuous line joins the median values for each frequency. *LTL*, lowest tested level.

WALTZING GUINEA PIGS

Twenty-four waltzing guinea pigs were conditioned for determinations of the threshold of hearing. An endeavour was made to obtain hearing thresholds as early as possible and to follow the development daily. Reliable hearing thresholds were recorded in 13 waltzers during the first week of life. Initially the thresholds were at the same level as, or slightly below that for the normals of similar age. Five of these waltzers showed a gradual improvement in hearing acuity and eventually hearing thresholds within the normal range were recorded. However the Preyer reflex thresholds in the waltzers, unlike those in the normal animals, were above the normal range and remained so throughout the post natal period (Figs. 3 and 10).

The difference between the Preyer reflex threshold in newborn normal and waltzing guinea pigs was the subject of a supplementary study performed on 162 animals, 68 waltzers and 94 normals. The thresholds were determined in the first 3 days of life. The median values and the semi-interquartile ranges for the two groups are presented in Table II. The difference between the median values was at least 5 dB at all frequencies except 3 kHz. There was no overlapping of the semi-interquartile ranges apart from a

Table I. *"The normal threshold of hearing for guinea pigs" median values and modal values chosen as a reference for further audiometry obtained from 30 normal adult animals*

SPL values. The lowest tested level as zero dB (re 0.0002 dyn/cm²); attenuator steps of 10 dB were used

Frequency (Hz)	125	250	500	1 000	1 500	2 000	3 000	4 000	8 000	12 000
Median value	13.3	12.1	7.0	2.1	2.1	2.1	2.1	2.1	12.1	12.1
Modal value	10	10	10	0	0	0	0	0	10	10

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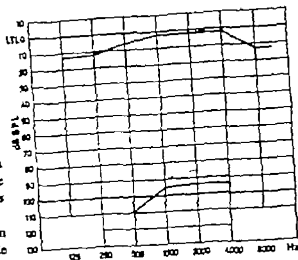


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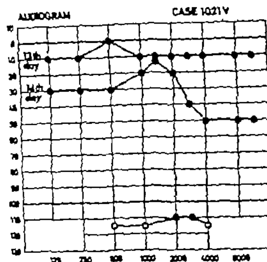


Fig. 4. First sudden impairment of hearing in waltzing guinea pig (no. 1021V). Audiograms obtained on 13th and 14th day of age. Note the reflex level, abnormally high but unchanged in spite of impairment of hearing threshold. Symbols, etc. as in Fig. 2.

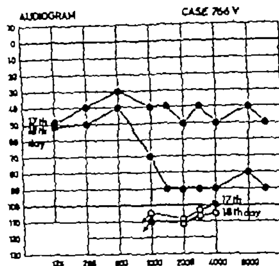
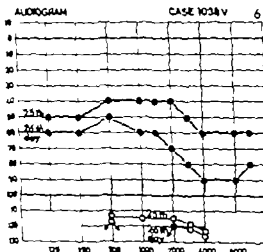


Fig. 5. Sudden hearing loss in middle intensity range in a waltzing guinea pig (no. 766V). Audiograms obtained on 17th and 18th day of age. Observe the much reduced gap between the hearing threshold and the reflex threshold (*recruitment*). Symbols, etc., as in Fig. 2.



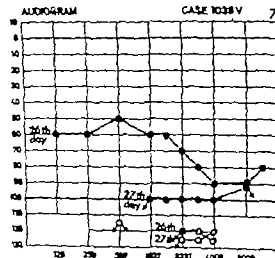


Fig. 6 and 7. Two steps of sudden hearing impairments without stable interval in waltzing guinea pig (no. 1038V). Audiograms obtained on 25th, 26th and 27th day of age. The figures illustrate how the high frequency range was first affected, and later the low frequency range. The relatively constant reflex levels reveal recruiting lesion. Symbols, etc., as in Fig. 2.

The Preyer reflex

The Preyer reflex threshold was always above the range for the normal guinea pigs, and it varied less than the level of hearing. Thus, even in those animals where the latter approached normal values the recorded reflex threshold was abnormally high. The subse

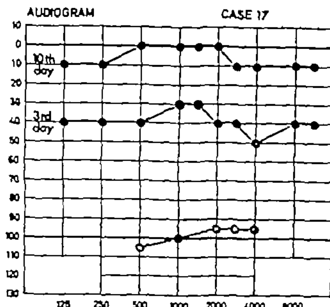


Fig 2 Audiograms obtained in a normal guinea pig (no. 17) on 3rd and 10th day of age showing post natal development of the hearing threshold. Filled circles, hearing threshold levels (free field determinations binaural hearing capacity) open circles, reflex threshold shaded area, normal range of the Preyer reflex threshold (see Fig. 1) Audiogram zero line represents normal threshold of hearing for guinea pigs

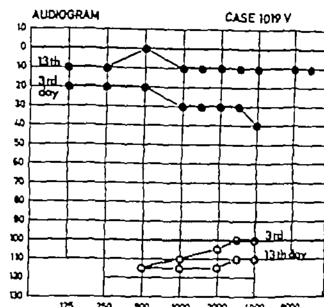


Fig 3 Audiograms obtained in a waltzing guinea pig (no. 1019V) on 3rd and 13th day of age, showing post natal maturation of hearing threshold. Note abnormally high reflex thresholds in spite of essentially normal threshold of hearing on 13th day Symbols, etc., as in Fig. 2

slight overlap at 3 kHz. The waltzing guinea pigs thus exhibited an elevated Preyer reflex threshold already in the immediate post natal period.

The subsequent course was followed in 7 waltzers until the hearing loss was total or the hearing threshold had dropped below 80 dB Of the further 17 waltzing guinea pigs, where this examination was begun but not completed some were sacrificed for morphologic examinations, others were discarded owing to sickness, and a few were lost because of too vigorous cooling during audiometry

In all the 24 waltzing guinea pigs the subsequent course was characterized by sudden stepwise, impairments of hearing acuity between which the hearing level was essentially stable These sudden impairments were initially confined to frequencies at and above 1 kHz, but later they appeared also in the low frequency region (Figs. 4 5 6 and 7). The stable intervals varied in duration from more than a week to nil. In a few animals a sudden loss of hearing was followed by a partial recovery of the previous threshold level lasting a few days. In some animals a gradual hearing loss of 10–20 dB was recorded for some days, but the sudden drops amounted to 30–50 dB or more No animal retained even residual hearing function beyond 42 days of age

Table II *Preyer reflex threshold levels in 94 normal and 68 waltzing guinea pigs obtained within 3 days of birth*

Median values and semi-interquartile ranges. Note difference in threshold already just after birth

	500 Hz			1 000 Hz			000 Hz			3 000 H			4 000 Hz		
	p_{25}	p_{50}	p_{75}	p_{25}	p_{50}	p_{75}	p_{25}	p_{50}	p_{75}	p_{25}	p_{50}	p_{75}	p_{25}	p_{50}	p_{75}
Normals ($n = 94$)	98.3	99.8	101.3	88.6	90.3	[illegible]	79.3	81.8	85.2	75.0	78.0	80.9	71.8	75.3	78.8
Waltzers ($n = 68$)	101.7	104.8	107.5	[illegible]	95.8	99.5	85.8	89.1	91.5	79.6	81.9	86.6	78.8	81.0	85.9

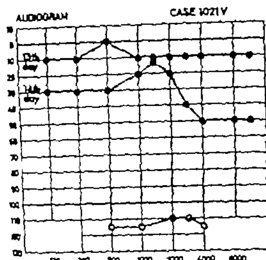


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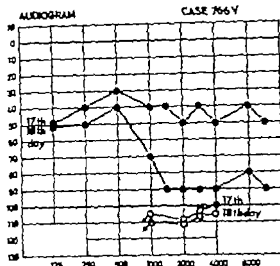
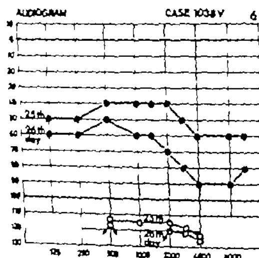


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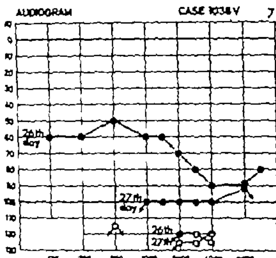


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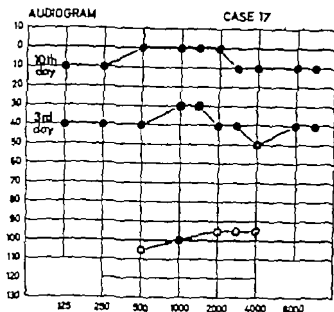


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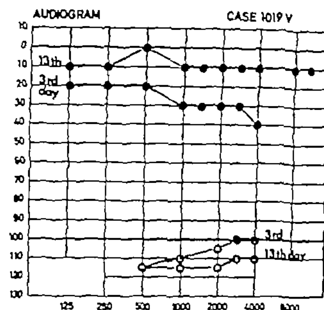


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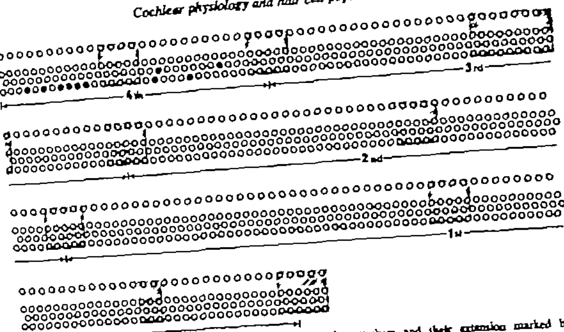
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A veraged cochleogram 1. Normal guinea pig (no. 1011 ala), prepared and cochleogram constructed as described (p. 6). Observe normally occurring slight hair-cell loss in the fourth turn. The turns are denoted by numbers and their extension marked by arrows. Shaded area represents approximate loss of hair cells from preparation. Cochleogram: see Appendix 1.

hair bundles. In the waltzing guinea pigs outer hair cells were missing also in the apical part, and to about the same extent. However in all these animals the other turns exhibited a greater or lesser hair-cell loss.

The mode of sensory cell degeneration in the waltzing guinea pigs has been described elsewhere (Ernstson, 1971 *b*). Abnormally configurated hair bundles were a common feature —at first on the inner hair cells and on the third row of the outer hair cells, and later in all the rows. The changes in hair-bundle configuration were most pronounced around the areas of maximum hair-cell loss. The degenerative changes in the sensory hairs are not indicated in the cochleograms, which, by definition, show only hair cells that are present or missing.

No 1013V illustrates the hair-cell population when the threshold of hearing had matured to within the normal range the reflex threshold was abnormally high (Fig. 10, averaged cochleogram 2, Appendix 2). The cochleogram revealed a random, in some areas quite extensive, hair cell loss, with a maximum in the basal part of the first turn. Another region where many hair cells were missing was the apical part of the second turn.

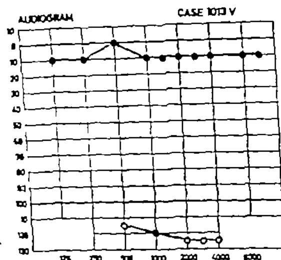


Fig. 10. Audiogram obtained immediately before sacrifice for hair-cell population survey in 14-day-old waltzing guinea pig (no. 1013V). Compare with averaged cochleogram 2 and Appendix 2. Symbols, etc., as in Fig. 2.

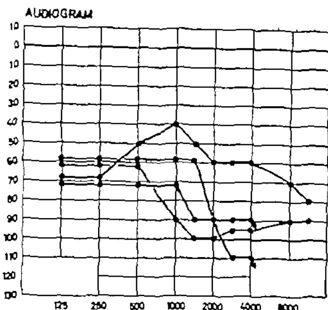


Fig 8 Audiograms showing hearing thresholds of 4 waltzing guinea pigs (nos. 43V 766V 1014V and 1019V) on the first day that the Preyer reflexes exceeded the audiometer capacity Thus, *an unelicitable Preyer reflex does not imply absence of hearing* Symbols, etc. as in Fig. 2.

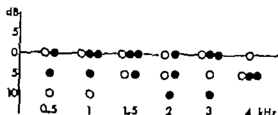


Fig 9 The middle-ear-muscles (MEM) reflex threshold level compared to the simultaneously determined Preyer reflex threshold level. For each point of measurement the MEM reflex was assigned the value of zero, and the corresponding level of the Preyer reflex threshold is shown (circles). Open circles: normal guinea pig filled circles. waltzing guinea pig.

quent course was, in general, characterized by a constant, or almost constant, reflex level, although the hearing threshold deteriorated (Figs. 4 and 5) Eventually the reflex threshold rose above the maximum capacity of the audiometer

In all the waltzing guinea pigs the gap between the hearing and the reflex thresholds was reduced during the course of the deterioration in hearing—in extreme cases to only 10–15 dB (Fig. 5) The hearing impairment was therefore invariably characterized by *recruitment* (Anderson & Wedenberg 1965 Ernstson, 1972, in press)

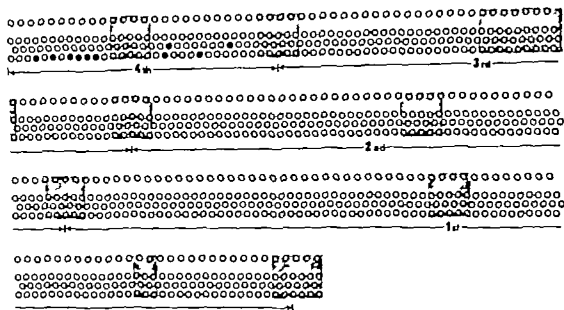
The threshold of hearing in 4 waltzers on the first day that the Preyer reflex could not be elicited at any frequency is presented in figure 8 *Accordingly* an animal with a normal Preyer reflex threshold level may or may not have a hearing loss, whereas an animal with an elevated Preyer reflex threshold level or abolished reflex may still have normal, or at least reasonable hearing sensitivity *Thus the Preyer reflex is useless as a test of hearing acuity*

Further evidence of the usefulness of the Preyer reflex test as a recruitment test in differential diagnosis was obtained from simultaneous determinations of the MEM and Preyer reflexes. Five guinea pigs were used, 2 normal and 3 waltzers. The two reflex threshold levels were found to coincide within 5 dB for all but 4 out of 28 points of measurements in the 4 exceptional *determinations the* difference was 10 dB or 2 attenuator steps (Fig. 9) There was thus a close agreement in the levels of the MEM and Preyer reflexes.

Hair cell population

A hair cell population survey was performed in 5 animals—one normal and 4 waltzing. In one of the latter no 1038V both cochleae were examined the results are presented in averaged cochleograms 1–6 and Appendices 1–5

In the normal guinea pig (no 1011) some outer hair cells were missing in the fourth turn and in the apical part of the third (averaged cochleogram 1 and *Appendix 1*) The other hair cells were present and normal in *appearance* as was the configuration of the



Averaged cochleogram 1. Normal guinea pig (no. 1011 sm), prepared and cochleogram constructed as described (p. 6). Observe normally occurring slight hair-cell loss in the fourth turn. The turns ar denoted by numbers and their extension marked by arrows. Shaded area represents approximative loss of hair cells *from preparation.* Cochleogram, see Appendix 1.

hair bundles. In the waltzing guinea pigs outer hair cells were missing also in the apical part, and to about the same extent. However in all these animals the other turns exhibited a greater or lesser hair-cell loss.

The mode of sensory cell degeneration in the waltzing guinea pigs has been described elsewhere (Ernstson, 1971 *b*). Abnormally configurated hair bundles were a common feature —at first on the inner hair cells and on the third row of the outer hair cells, and later in all the rows. The changes in hair-bundle configuration were most pronounced around the areas of maximum hair-cell loss. The degenerative changes in the sensory hairs are not indicated in the cochleograms, which, by definition, show only hair cells that are present or missing.

No 1013V illustrates the hair-cell population when the threshold of hearing had matured to within the *normal range* the reflex threshold was abnormally high (Fig. 10, averaged cochleogram 2, Appendix 2). The cochleogram revealed a random, in some areas quite extensive, hair cell loss, with a maximum in the basal part of the first turn. Another region where many hair cells were missing was the apical part of the second turn.

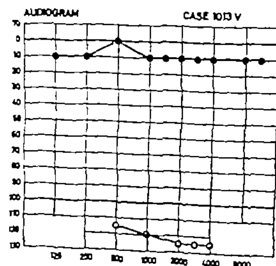
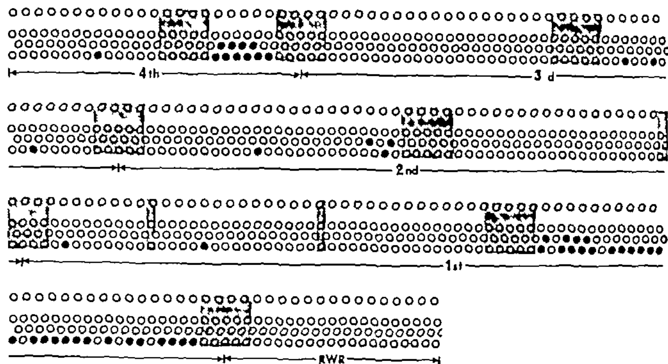
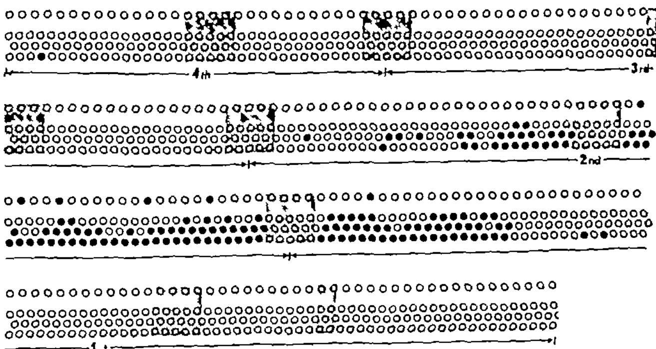


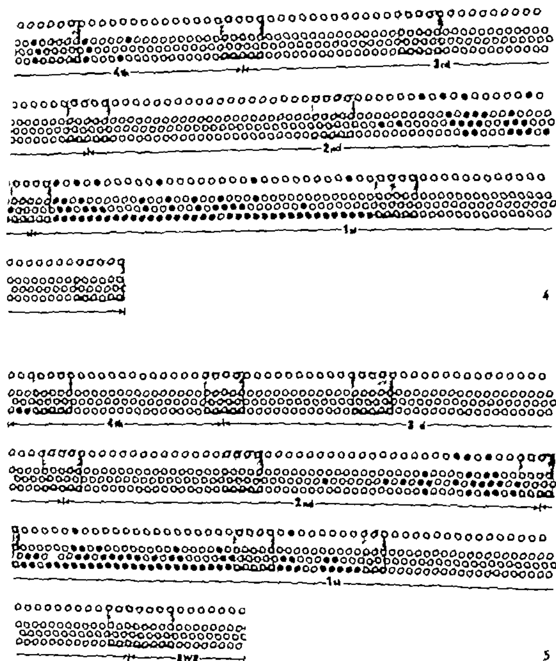
Fig. 10. Audiogram obtained immediately before sacrifice for hair-cell population survey in a 14-day-old waltzing guinea pig (no. 1013V). Compare with averaged cochleogram 2 and Appendix 2. Symbols, etc., as in Fig. 2.



Averaged cochleogram 2 Immediately prior to preparation the animal (*no. 1013V dx*) *exhibited a normal* threshold of hearing but an elevated reflex threshold (Fig. 10). *Many third-row* outer hair cells missing in the basal part of 1st turn a random loss *of a few outer hair cells in the other turns. RWR*, round-window region Other markings as in averaged cochleogram 1 Cochleogram, see Appendix –



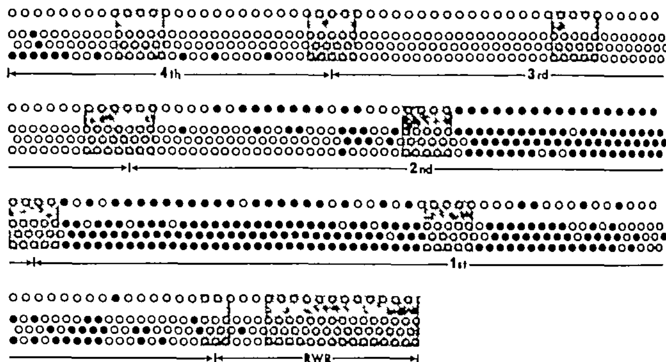
A eraged cochleogram 3 No. 12V sin. Hearing threshold at the 70–80 dB level immediately prior to preparation elevated reflex threshold and reduced gap between hearing and reflex thresholds (Fig. 11). A considerable number of outer hair cells lost at the transition of the 1st and 2nd turns a few inner hair cells also missing. Cochleogram see Appendix 3 *Markings as in a eraged cochleogram –*



Averaged cochleograms 4 and 5. In no. 1038V both organs of Corti were studied for comparison. Hearing function, see Fig. 12. Obvious elicitable reflex in spite of almost total deafness. The hair-cell loss in extent and localization are practically equal in the two ears, and similar to those in no. 12V. Few inner hair cells missing. Cochleogram of left ear: see Appendix 4. Markings as in averaged cochleogram 2.

Damage was most extensive in the third row of the outer hair cells, whereas very few inner hair cells were lost.

In no. 12V the reflex threshold level was comparable to that in the previous animal, but the hearing threshold was recorded at the



Averaged cochleogram 6 Here (no. 43V dx) the reflex was unelicitable 7 days prior to preparation the hearing threshold (Fig. 13) comparable to that in no. 1038V In the apical half of the 1st turn and the basal part of the 2nd turn few outer hair cells present: observe the corresponding inner hair-cell loss. Cochleogram, see Appendix 5 Markings as in averaged cochleogram 2.

70 dB level (Fig. 11 averaged cochleogram 3 and Appendix 3) There was a marked loss of outer hair cells in the first and second turns. In small areas all the outer hair cells were missing a few inner hair cells had also disappeared.

In no. 1038V the hearing threshold (Fig. 12) could be obtained for only 6 test frequencies

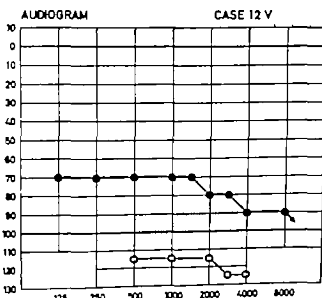


Fig. 11 Audiogram obtained immediately before sacrifice for hair-cell population survey in a 20-day-old *waltzing guinea pig (no. 12V).* Compare with averaged cochleogram 3 and Appendix 3 Symbols, etc., as in Fig. 2.

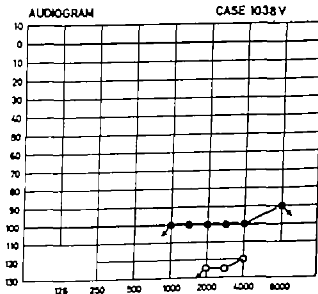


Fig. 12 Audiogram obtained immediately before sacrifice for hair-cell population survey in a 27-day-old waltzing guinea pig (no. 1038V). Compare with averaged cochleograms *4 & 5 and Appendix 4 Symbols*, etc., as in Fig. 2.

and the reflex threshold for only 3. There was a hair cell loss (Appendix 4) similar to that in the previous animal. The extent of the depopulation was practically the same in the two ears (averaged cochleograms 4 and 5).

No. 43V with a threshold of hearing comparable to that in no. 1038V showed an increased depopulation of hair cells in the organ of Corti (Fig. 13, averaged cochleogram 6, Appendix 5). Not only was there total loss of the outer hair cells in large areas but the corresponding inner hair cells were also missing. In this guinea pig the Preyer reflex was unelicitable already 7 days before sacrifice.

It should be observed that in all the waltzers, including nos. 1038V and 43V with their almost total loss of hearing, large parts of the cochleae were still populated with apparently viable hair cells. Even in no. 43V the third turn was essentially normal in this respect.

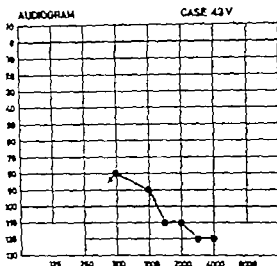


Fig. 13. Audiogram obtained immediately before sacrifice for hair-cell population survey in a 31-day-old waltzing guinea pig (no. 43V). Compare with averaged cochleogram 6 and Appendix 5. Symbols, etc., as in Fig. 2.

Discussion

The audiometric technique used in the guinea pigs was reasonably easy to perform and gave reproducible results. As the hearing threshold tests could be completed in less than half an hour they could be carried out almost hourly as previously shown (Ernstson, 1972, in press), and the method was therefore eminently suitable for evaluating even quite rapid changes in hearing acuity. Furthermore, once conditioned the animals retained their ability to respond appropriately for more than a year: supplementary conditioning was thus unnecessary.

Though there is now an abundance of studies of various aspects of the guinea-pig cochlea, remarkably little is known about this animal's normal threshold of hearing. In a pilot study Anderson and Wedenberg (1965) examined the hearing threshold in 2 guinea pigs. In the present study the normal hearing threshold was determined in 30 adult guinea pigs in order to provide a reference in further audiometric work. In the audiograms presented the SPL thresholds were converted so that the zero line actually represents the "normal threshold of hearing for guinea pigs". It should be observed that the lowest tested value was zero dB (re 0.0002 dyn/cm²). There was a distinct resemblance between the median hearing thresholds in guinea pigs and the normal hearing thresholds in man for a similar mode of tone presentation (free field, ISO R 226). As the hearing tests were performed in free field, the thresholds represent the binaural hearing capacity. No attempt was made to establish the total frequency range of hearing.

The range of the normal Preyer reflex thresholds was determined in the 30 normal adult guinea pigs. The median threshold level

was calculated and modal values were also chosen but in the audiograms the total range was used (shaded area) as this information proved more appropriate

Somewhat unexpected was the evidence of functional immaturity of hearing just after birth none of the 4 normal guinea pigs exhibited normal hearing thresholds in the first 10 days of life. The presence of a conductive impairment due to for instance middle ear fluid was ruled out by the normal reflex thresholds. Electron microscopic examination has disclosed no morphologic signs of post natal immaturity in the organ of Corti of the normal guinea pig (Ernstson, 1971 *b*) Vestibular tests in newborn and adult normal guinea pigs have yielded identical results (Ernstson 1971 *a*) Whether this progressive improvement in hearing acuity is indicative of a functional maturation process in the end-organ *per se* or whether it reflects an increasing adaptation of the animal to environmental stimuli remains an open question

The Preyer reflex has been widely accepted and used as a hearing test in the guinea pig. In his original investigation Preyer (1881) demonstrated the "maschinenmässigen Sicherheit of the reflex movements elicited by strong sound stimuli. However he also observed the ability of the newborn guinea pig to perceive the soft, rather low intensity voice of the mother "auch wenn diese nur ganz leise und abgebrochen schnurrt Preyer thus recognized the difference between supra and hearing threshold reactions. This difference has been largely overlooked, and the Preyer reflex has often been relied upon as the sole hearing test. The present investigation has shown that there is no parallel between the hearing threshold and the Preyer reflex threshold, and that *the Preyer reflex thus cannot be used as a test of hearing acuity* This affords an explanation of the discrepancies in the results of several earlier studies. On the other hand, the close agreement between the MEM and the Preyer *reflex thresholds in the* guinea pig justifies parallels with the stapedius reflex test in man. The accumulated knowledge from hearing tests in man, especially that relating to topical localization (recruitment) may therefore now be applied to the guinea pig.

It is well known that in many mutants of various species there is a gradual deterioration of the hearing acuity from birth. Even so determinations of the hearing threshold in animals with inherited cochlear lesions are scarce (see Anderson et al. 1968) Whereas the morphologic aspects of the inner ear degeneration has been extensively studied in many mutants (see Altmann, *1950* Deol, *1968*), the physiologic features such as the course and character of the hearing function impairment have received little attention

A study of the physiologic features was undertaken on our strain of waltzing guinea pigs. Even post natally the waltzers exhibited an elevated threshold of hearing and an abnormally high reflex threshold. In some of the animals the hearing acuity gradually matured to near normal, but the reflex thresholds remained above the normal range. The hearing usually stayed at a stable elevated level until later impairment

Whether the hearing acuity matured or not the subsequent course was characterized by sudden impairments in the hearing threshold separated by essentially stable intermissions. The impairments were initially confined to the higher frequencies, but later on the low frequency range was also affected. In some cases a partial recovery of the previous threshold level was noted. In some guinea pigs there was a slowly progressing but temporary elevation of the hearing threshold. The immediate cause of the sudden impairments is obscure and for obvious reasons cannot be ascertained by conventional morphologic methods.

The course of the hearing loss was invariably characterized by a reduced gap between the hearing and the reflex thresholds—that *is to say by recruitment* A topical diagnosis, in which the lesion was localized to the coch-

lea, was therefore performed and fully substantiated by the morphologic investigation (Ernstson 1971 *b*).

The results of the hair cell population survey was to some extent consistent with the audiograms—the more extensive the hair-cell loss the greater the hearing impairment. In contrast to the rather sharply demarcated cochlear hair cell damage caused by for example, ototoxic antibiotics (see Kohonen, 1965 Ostyn & Tyberghein, 1968, Stebbins et al., 1969), the depopulation pattern in the endogenous damage in the waltzing guinea pigs was considerably more diffuse. Furthermore, a large proportion of the sensory cells displayed changes of the hair-cell top and would not be expected to react to stimuli in the same manner as normal neuroepithelium, and hence there would hardly be any close consistency between the audiograms and the cochleograms. The hair-cell loss in the apical part of the cochlea is shown to be a regular feature (Stockwell et al. 1969).

The cyto-architectural division of the organ of Corti into outer and inner hair cells may reflect differences in properties, such as sensitivity The inner hair cells exhibit a morphologic resemblance to the phylogenetic older vestibular neuroepithelium (Wersäll & Flock, 1967). The animal in the present study whose Preyer reflex had been extinguished 7 days prior to sacrifice (no. 43V) had fewer inner hair cells over a wide area than the one (no 1038V) whose reflex could still be elicited, the impairment of the hearing threshold in these two animals was similar

Though not conclusively proved, it seems not unlikely that, being a more primitive and suprathreshold reaction, the Preyer reflex is initiated by the inner hair cells. The threshold of these would then be equal to the reflex threshold level. The outer rows of hair cells might respond to stimuli at the hearing threshold level and at increasing intensity until eventually the inner hair cells are stimulated and the reflex elicited.

Summary

The normal threshold of hearing for guinea pigs was established by the method evolved by Anderson & Wedenberg (1965). The course of the hearing loss was followed in a strain of the waltzing guinea pig with a dominant mode of inheritance of the anomaly In normal animals and some waltzers a post-natal maturation of hearing acuity was observed. The inherited hearing loss in the waltzers progressed by sudden steps separated by stable intervals of varying length. The high-frequency range was the first to be affected, later the low-frequency range. Throughout the period of the hearing loss there was a characteristic reduction of the gap between the hearing and the Preyer reflex thresholds, i.e. *recruitment*. The Preyer reflex threshold was abnormally high in the waltzers—even just after birth. The levels of the middle-ear-muscles reflex and the *Preyer reflex thresholds* were found to coincide in normal and in waltzing guinea pigs. It is concluded that whereas the Preyer reflex test does not disclose the hearing acuity it can be used as a topical test in differential diagnosis (recruitment). The parallels to the stapedius reflex test in man are obvious.

A hair-cell population study disclosed a progressive rather random loss of sensory cells, which was most pronounced in the first and later the second turns. The depopulation pattern was to some extent consistent with the audiograms. A tentative hypothesis is advanced regarding the role of the inner hair cells in eliciting the reflex.

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Zusammenfassung

Die normale Hörschwelle für Meerschweinchen wurde mittels der Methode von Anderson und Wedenberg (1965) festgestellt. Der Verlauf des genetisch ausgelösten Hörverlustes wurde bei einem erblich dominanten Stamm waltzender Meerschweinchen untersucht. Bei normalen und einigen waltzenden Tieren wurde eine post natale Verbesserung des Hörvermögens gefunden. Der erbliche Hörverlust der waltzenden Tiere verschlimmerte sich sprungartig mit stabilen Intervallen bei unterschiedlicher Dauer. Anfangs wurde das hoch frequente, später auch das *tief frequente Gebiet getroffen*. Ganz charakteristisch während des Degenerationsvorganges war der verminderte Abstand zwischen der Hörschwelle und der Preyer Reflexschwelle was Recruitment zeigt. Die Preyer Reflexschwelle war bei den Waltzenden immer pathologisch erhöht, auch in der unmittelbaren post natalen Periode. Eine Übereinstimmung der Schwellen der Mittelohrmuskelreflexe und der Preyer Reflexe wurde an normalen und an waltzenden Tieren gezeigt. Es konnte klar gelegt werden, dass, obwohl der Preyer Reflex nicht zur quantitativen Bestimmung der Hörfunktion anwendbar ist, kann er doch für *differentialdiagnostische* Zwecke verwendet werden (Recruitment). Die Ähnlichkeit mit dem *Stapediusreflex beim Menschen ist auf*fallend.

Übersichtliche Untersuchungen an den Haarzellen des Cortischen Organs ergaben einem ziemlich variablen Verlust der Haarzellen, der sich von einer *Prädilektionsstelle* in den ersten und zweiten Schneckenwindungen mit zunehmendem Alter ausbreitete. Diese Vorgang war mit den Audiogrammen in gewisser Hinsicht übereinstimmend. Eine Hypothese über die Funktion der inneren Haarzellen wird präsentiert.

Acknowledgment

My sincere thanks are due to Henry Anderson, Assistant Professor in whose laboratory and under whose guidance and inspiring encouragement this work was carried out. Professor Bengt Barr, Professor Erik Wedenberg and Elis Pettersson, Engineer furnished invaluable support throughout the course of the work.

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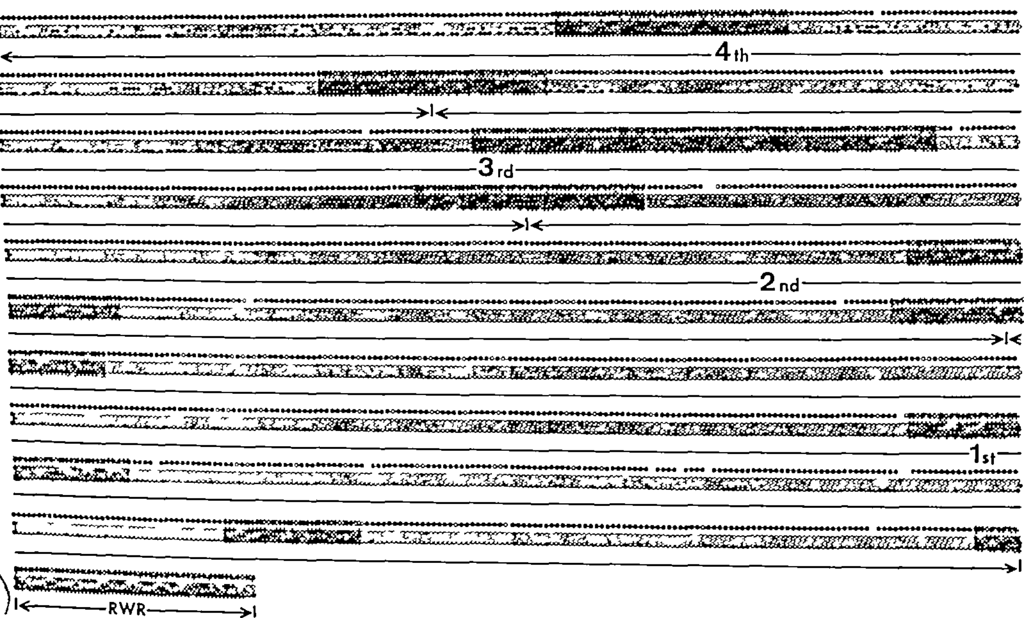
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Appendix I. No. 1011 sin. Cochleogram constructed as described in text (p. 6). See averaged cochleogram 1. The turns are numbered and their extension marked by arrows. *RWR* round-window region. Shaded area denotes approximate loss of hair cells from preparation.

4th

3rd

2nd

1st

RWR

h 2 No. 1013V dx. See averaged cochleogram — Markings as in Append x 1

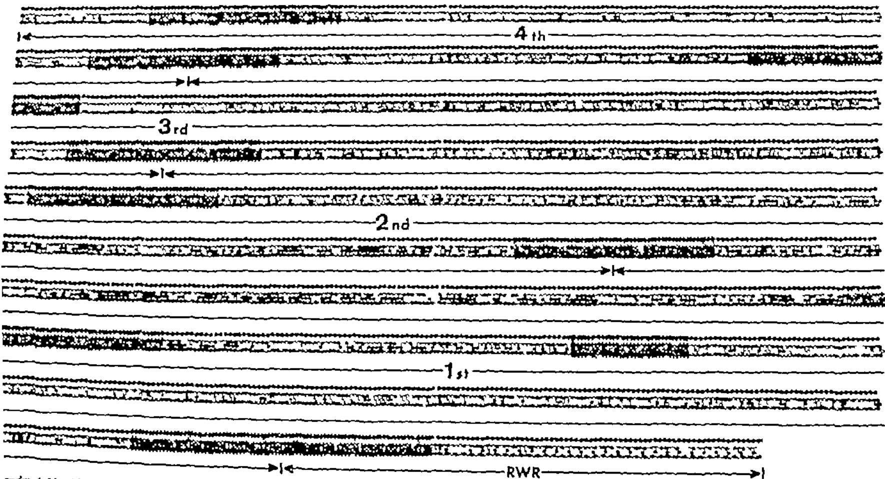
4th

3rd

2nd

1st

Appendix 3 No 12V sin. See averaged cochleogram 3 Markings as in Appendix 1



Cndir 4 No. 103EV ain. See veraged cochleogram 5 Markings as in Appendix 1

4th

3rd

2nd

1st

RWR

Append 5 No 43V dx See a craged cochleogram 6 Markings as in Appendix 1

4th

3rd

2nd

1st

RWR

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Acta

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SUPPLEMENT 298

A Florilegium of Experiments on Directional Hearing

BY

J DONALD HARRIS

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A Florilegium of Experiments on Directional Hearing

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The University of Connecticut, Connecticut, USA

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CONTENTS

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Introduction

The question of how efficiently man can localize a sound source in space received its first experimental answer using acoustical controls acceptable by modern standards in the study of Stevens & Newman (1936). Their results were recently popularized widely by Stevens & Warshofsky (1965). It appeared that one could not point to a sound source even in the frontal quadrant with less than a 3–4 average error at the better frequencies, while at other frequencies (including those at which the ear is most acute!) the error commonly reached 9–12 and at other quadrants was even greater But if we could not really do better than that, the race could hardly have survived and of course it was clear to the authors that information had existed for many decades on sound localization as a function separately of interaural differences in intensity phase and time-of-arrival on the basis of which one would predict a minimum audible angle (m.a.a.) of only a very few degrees at any frequency or azimuth.

This paradox was finally resolved in Stevens laboratory by Mills (1958), whose widely-cited paper revealed that the m.a.a. in the frontal quadrant (horizontal plane) was indeed of the order of 1–4 at any frequency and that the general shape of the curve of the m.a.a. s frequency was predictable from what was then known of interaural intensity and phase (Mills, 1960).

A few questions remain secondary to the major one solved by Mills classic study First of all, its importance makes a direct replication very much in order There is a perturbation in the data at 1.6 kHz such that the m.a.a. seems unaccountably poor in the data of Stevens & Newman a similar perturbation was an octave or more higher in frequency Why indeed should there be any frequency *effect* at all? Again, it appeared that when the sound sources came from a region about 60 off the midline, the m.a.a. deteriorated sharply But it is not our common experience that in daily life the errors at such angles are crippling.

Beyond checking Mills data, and perhaps exploring some seeming discrepancies, it would be important to know whether other types of directionality tasks would yield the same general precision of m.a.a. For example, Sandel et al. (1955) performed a directionality study using a different psychophysical procedure (an acoustical pointer") and their data have been recomputed by Mills (1960) to yield m.a.a. quite comparable to those of Mills; but only one azimuth was used. Generality of application would of course be improved if it could be shown that m.a.a. is relatively independent of method.

Going beyond the determination of m.a.a. at a selection of azimuths, elevations, and frequencies are broader questions. We wish to know what part of directionality can be accounted for by the physical characteristics of *S*'s head and pinnae, what aspect or aspects of interaural acoustic differences contribute to directionality and if more than one, we wish to know what portion is contributed by each aspect, whether intensity phase, time-of-arrival, etc.

The following studies were initiated in an attempt to redetermine normal human m.a.a. in the horizontal plane by several techniques, to throw some light on the physical conditions conducive to good directionality and to begin to assess the relative contributions of these.

Introduction

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The following studies were initiated in an attempt to redetermine normal human m.a.a. in the horizontal plane by several techniques, to throw some light on the physical conditions conducive to good directionality and to begin to assess the relative contributions of these.

...e two experiments, in view of the fact that many details of the apparatus and procedure differed, and that relatively few *Ss* were available for the many arduous sessions needed in the Method of Constants. We may take it as a generality then, that the normal human ear has an m.a.a. of about 1–2 through the audible range.

Mills' demonstration of a slight deterioration at 1.6 kHz was corroborated. His explanation, however must be re-exammed (see below) that 1 6 kHz is a frequency at which the interaural phase cue is becoming inoperative while the interaural intensity cue is not yet fully useful. It will be seen that the interaural intensity cue is fully sufficient to yield judgments of minimum sidedness, while the even more powerful interaural time cue is as available at 1.6 kHz as at any other frequency region.

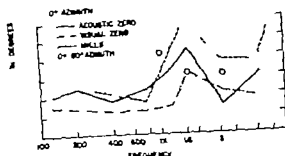


Fig. 1 Minimum audible angle as a function of frequency at 0° and 60° azimuth, for both an acoustic and a visual zero (including comparable data from Mills (1958) using an acoustic "zero").

Exper II Effect of Minimizing Head Movement

Although in Exper I the use of a bite board to fix the head, and a brief (1/4-sec) stimulus duration, reduced to what was thought a negligible effect of rotating the skull on the atlas, nevertheless some possibility existed that head movements may have played a role in the m.a.a. It seemed desirable to eliminate the possibility altogether

APPARATUS AND PROCEDURE

Ss, apparatus, and procedures were essentially the same as in Exper I, but the stimuli were presented to *S* by earphone and tape recorder The tape was prepared with the use of an immobile artificial head (see Fig. 2) inserted exactly a *S*' head in the anechoic chamber and picking up just such stimuli as in Exper I by way of high-fidelity equipment beginning with microphones in the position of the ear drums As before, *S* had to respond with L–R lateralization judgments.

The artificial head consisted of a balsa wood core carved by a sculptor with a layer of softish rubber about 1/4 to 1/2 inch thick. Pinnae of the same rubber transferred by a plaster of Paris technique from real heads were made, one from an adult with pinnae 2.5 inches in the vertical dimension, the other from a 6-year-old boy (the writer appreciates the courtesy of Todd N Harris in thus lending me his ears). Either pair could be pinned to the head over the meati and faired in with modelling clay to present an entirely normal appearance. The meati were simply wooden plugs 1.25 inches long with center-drilled 5/16-inch holes, the inner surface funnelled slightly to mate to Western Electric 640AA microphones (the courtesy of the Underwater Sound Laboratory in lending one of these, and the SubMedResLab the other is acknowledged). The outputs were led to Western Electro-Acoustic Lab preamplifiers filters, and VTVMs calibrated in SPL.

With both circuits carefully balanced for a single source at 0 azimuth, 2-channel recordings of typical 1/4-sec stimuli at 0.8 and 1.2 kHz were made on an Ampex PR 10-2 unit,

Exper I M A A with Acoustic "Zero"

SUBJECTS

One female (age 23) and three men (aged 32–44) were used all with normal hearing for the frequencies examined.

APPARATUS

A pair of 15 ft aluminum I-beams were supported one above the other in an anechoic chamber lined with 44-inch fiberglass wedges 32.5 ft from tip to tip. Each beam formed a railroad track marked off in inches to support an aluminum cart with nylon wheels, practically noiseless as it was pulled L or R along the rails by a variable speed reversible DC motor nylon pulleys, and steel thread system. On each cart was fixed a Jansen 15-inch triaxial loudspeaker and cabinet, the top cabinet upside down so that the axes of the speakers would be as close as physically possible namely 19 inches, to which S is relatively very insensitive

Conventional commercial oscillators, electronic switches and timers, filters, and attenuators controlled the frequencies, sound pressure levels (SPLs) and timing of the speaker outputs. A frequency meter and VTVM maintained frequency and intensity calibration A wave analyzer was used to examine the stimulus at certain times, but with the moderate SPLs (about 60 db SPL) and limited frequencies (0 125–8 kHz) employed distortion was never a problem.

PROCEDURE

S sat blindfolded in a comfortable chair fitted with a dental bite board, his interaural axis 10 ft from the middle of the rails. The bottom speaker was keyed on for a time (either 1/4 or 3 sec) always in S's median sagittal plane (0 azimuth) then after 1 sec the top speaker was keyed on for the same time. Rise-fall time was always 10 msec. S simply had to judge whether the second speaker seemed L or R of the first. Ten judgments were made by each S at each of a selection of distances (convertible by simple geometry to degrees azimuth) The distances were chosen at random within the range to blanket S's interval of uncertainty of directionality The 75%-correct distance was found graphically by plotting scores on probit paper An extraneous noise after every trial masked any sounds made by moving the top speaker to a new location between trials. Knowledge of trial results was not given except as specified below

RESULTS AND DISCUSSION

Table I shows the individual and mean m.a.a. when the stimulus duration was 1/4 sec. For all frequencies the m.a.a. was about 1–1.5 with the exception of 1 6 kHz where all Ss yielded their worst m a a. by roughly 1

A comparision of the mean data with those of Mills (1958) (see Fig 1) shows quite good agreement for this sort of judgment between

Table I *Minimum audible angle in degrees for pure tones using an acoustic ero at 0 A imuth*
Temporal pattern 1/4 sec on — 1 sec off — 1/4 sec on

	Frequency in kHz							
Subjects	0 125	0.2	0.4	0.8	1	1 6	3	6 4
RLS	0.81	1.34	0 48	1 6[illegible]	2.48	[illegible]	0.6[illegible]	1 57
RLM	0.95	1.29	1 14	1 19	1 81	[illegible]48	0.68	[illegible]39
JDH	1 14	1.34	1 38	1.57	1.53	1 67	0.95	1 34
EBB	1 10	1 00	0.6[illegible]	0.57	1 00	1 67	0 68	0 76
Mean	1 00	1.[illegible]4	0 91	1 [illegible]4	1 71	[illegible]26	0.73	1 51

Table III. *Minimum audible angle in degrees for pure tones using an acoustic zero at 0° azimuth*
Note: Head movements emphasized. 3 sec on — 1 sec off — 3 sec on

	Frequency in KHz							
Subjects	0.125	0.25	0.4	0.8	1.25	1.6	3.2	6.4
RLS	1.2	0.8	0.3	2.1	2.1	0.9	0.5	0.9
RLM	1.8	0.8	0.5	0.9	1.7	0.6	0.4	1.1
ERB	2.4	2.0	0.35	0.4	1.7	0.7	1.4	2.3

render more likely the efficiency of cumulative sampling.

RESULTS AND DISCUSSION

Table III gives the data for the three Ss. A detailed comparison with the germane figures in Table I reveals that no especial trend exists, except that the deterioration of m.a.a. at 1.6 kHz with the 1/4-sec duration is not seen with the 3-sec duration. It would seem that a fuller time sampling overcomes any disadvantage which this frequency may possess on the first quick look" at the stimulus.

Exper IV Effect of Azimuth

When Mills required his Ss to judge directionality with the first of two stimuli 60 off midline all m.a.a. deteriorated, but especially those in the region centered again on 1.6 kHz. We therefore repeated Exper III but at 30 azimuth for one S as well as at 60 for all Ss at 1 .5–3.2 kHz.

When the loudspeakers were set at 30 azimuth, it was immediately noted that even though the two loudspeakers were lined up on the same azimuth, the difference of a few degrees in the vertical dimension sometimes gave the impression that the second speaker (upper) was at a slightly different azimuth than the first speaker (lower) due to the geometry of the room and of its contents, however meager The upper loudspeaker was therefore set at 30° (or 60) and the lower moved L–R at the beginning of each experimental session to a new azimuth which

Table IV *Minimum audible angle in degrees for pure tones using an acoustic zero at 30° and at 60° azimuth*

Note Head movements emphasized. 3 sec on — 1 sec off — 3 sec on

	Frequency in KHz							
	0 125	0.2	0 4	0.8	1.25	1.6	3.2	6.4
30 Azimuth								
Subject JDH	1 0	0 9	0.5	1.2	1 1	1.5	1.9	2._
60 Azimuth								
Subjects								
RLS					_9	1.8	1.8	
RLM					2._	1.4	—	
JDH					2.0	1.4	1.9	
LBB					1 4	3.0	1.7	
Mean at 60					2.(	1.9	1.8	

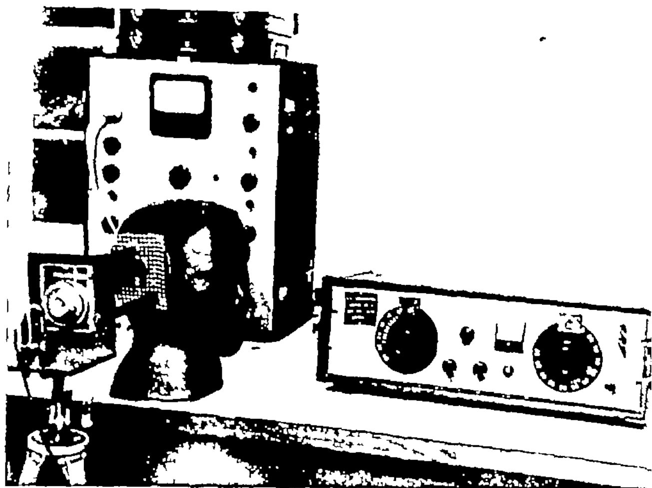


Fig 2. The Shilling artificial head (Fig. 2 in Rosen et al. 1964).

and this tape later played to *Ss* wearing a matched pair of Beyer DT-48 earphones.

RESULTS AND DISCUSSION

The stimulated m a.a. from the tapes are in Table II The means are indistinguishable from those of Table I and we may conclude that head movements to sample directionality in the front quadrant are not necessary to yield good m a.a. (though obviously they may well be useful in solving front-back confusions, and perhaps in other situations)

Table II *Minimum audible angle in degree simulated by magnetic tape*

Note. Head movements minimized

	Frequency in kHz (Stimulus at 60 DB SPL)		
Subjects	0.8	1.2	White noise
RLS	2.7	1.5	[illegible].0
RLM	1 8	[illegible].8	4.0
JDH	1 2	0.9	1 4
EBB	[illegible].5	1 4	0.9
Mean	[illegible].05	1 65	[illegible].1

Exper III Effect of Maximizing Head Movement

The question arose whether head movements would have improved the m a.a of Exper I had *S* not used a bite board and had the stimulus not been so brief Accordingly the bite board was removed and three *Ss* were encouraged to sample directionality by rotating the head as each might think best the duration of each tone was lengthened to 3 sec to

Table III. Minimum audible angle in degrees for pure tones using an acoustic zero at 0° azimuth

Note: Head movements emphasized: 3 sec on — 1 sec off — 3 sec on

	Frequency in KHz							
Subjects	0.125	0.25	0.4	0.8	1.25	1.6	3.2	6.4
RLS	1.2	0.8	0.3	2.1	2.1	0.9	0.9	0.9
RLM	1.8	0.8	0.9	0.9	1.7	0.6	0.4	1.1
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render more likely the efficiency of cumulative sampling.

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Table IV Minimum audible angle in degrees for pure tones using an acoustic zero at 30° and at 60° azimuth

Note Head movements emphasized: 3 sec on — 1 sec off — 3 sec on

	Frequency in kHz							
	0.125	0	0.4	0.8	1.25	1.6	3.2	6.4
30 Azimuth								
Subject JDH	1.0	0.9	0.5	1.[illegible]	1.1	1.5	1.9	2.2
60 Azimuth								
Subjects								
RLS					.9	1.8	1.8	
RLM					2.2	1.4	—	
JDH					.0	1.4	1.5	
ERB					1.4	3.0	1.7	
Mean at 60					2.1	1.9	1.8	

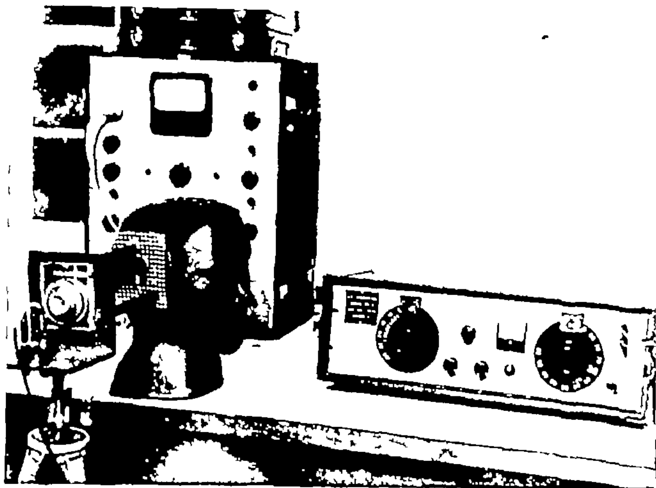


Fig 2 The Shilling artificial head (Fig. _ in Rosen et al. 1964)

and this tape later played to *Ss* wearing a matched pair of Beyer DT-48 earphones.

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Table II *Minimum audible angle in degrees simulated by magnetic tape*

Note Head movements minimized

	Frequency in KHz (Stimulus at 60 DB SPL)		
Subjects	0.8	1._	White noise
RLS	_7	1.5	_0
RLM	1 8	_8	4 0
JDH	1	0.9	1 4
EBB	_5	1 4	0 9
Mean	2.05	1 65	2.1

Exper III Effect of Maximizing Head Movement

The question arose whether head movements would have improved the m a.a. of Exper I had S not used a bite board and had the stim ulus not been so brief Accordingly the bite board was removed and three *Ss* were encour aged to sample directionality by rotating the head as each might think best the dura tion of each tone was lengthened to 3 sec to

Table V *Minimum audible angle for left-right directionality of a single moving sound*

Subjects	Frequency in KHz 0.8	1.6	3.2	6.4
RLS	3.7	6.5	7.5	—
RLM	2.5	1.4	2.3	0.9
JDH	4.4	4.5*	3.6	2.5

* Necessary to use a bite board to stabilize data.

judgment being easy and precise for *S* it was in fact easy but imprecise. Table V shows the (to us) surprisingly large m.a.a. Note only that performance at 1.6 kHz was no better or worse than at other frequency regions.

The imprecision of the moving-source m.a.a. may be related to the fact that in the nature of the situation both stimulus and *S*'s head were moving, in which case *S* would be uncertain as to the effect of the movement of the source *per se*. With one *S*, indeed, at 1.6 kHz the head had to be stabilized by a bite board before real consistency appeared in the data.

Exper VI M.A.A with a Visual Zero

In daily life one of our commonest experiences is to coordinate the eyes with the ears. We seek to turn the head toward a source of sound of unknown directionality and form a congruence of meaning between the two senses. As the head is turned on the atlas so that the sound source is in the median sagittal plane, the interaural conditions are at maximum symmetry and the eyes also are symmetrically innervated and disposed. Using the direction of regard as a visual pointer then we felt *S* might be expected to say whether when his visual conditions were symmetrical, an asymmetry of the interaural conditions might be efficiently detected. Accordingly we put this hypothesis to test.

APPARATUS AND PROCEDURE

An acoustically transparent but visually opaque cloth was stretched on a wooden frame to obscure all apparatus. A vertical stripe (a length of white twine) was stretched also on the frame passing through the 0 azimuth. Experimenter positioned the speaker L or R of the midline at random, without acoustic or other cues, then alerted *S* to look fixedly at the stripe and listen for the ensuing 3-sec tone burst. Judgment L or R was called for

RESULTS AND DISCUSSION

Table VI shows that, contrary to our expectation, m.a.a. with only a visual zero is even

Table VI *Minimum audible angle in degrees for pure tones using a visual zero*

Note Temporal pattern. Tone on for 3 sec

Subjects	Frequency in KHz 0.125	0.4	0.8	1.25	1.6	3.2	6.4
RLS	0.2	0.4	0.[illegible]	0.7	1.6	1.34	1.4
RLM	0.6	0.7	0.4	0.45	1.1	0.4	0.7
JDH	0.7	1.0	1.05	1.35	2.6	1.3	0.6
LBB	1.2	1.0	1.0	1.3	1.6	1.7	0.9
Mean	0.67	0.77	0.66	0.9*	1.72	1.17	0.9

* Necessary to use a bite board to stabilize data.

seemed to S to be exactly that of the first sound source. Thereafter the second loud speaker was positioned around this new "Zero On successive settings the range of the new "Zero with respect to the true azimuth, was 12 inches L on one occasion at 1 25 kHz. Usually it was 2 inches or less. This asymmetric and unpredictable constant error was seldom found using the median sagittal plane, and was found somewhat more pronounced at the 60 azimuth than at the 30 However even at the 60 azimuth it never exceeded 12 inches L or R. For all sittings at 30 and 60 azimuth care was taken to control for this constant error

RESULTS AND DISCUSSION

A comparison of data for S JDH tested at 30 with his data at 0 (Table I) shows no tendency for m a.a to deteriorate with an off midline Zero Evidently with the constant error eliminated 30 azimuth is as good as 0° if not at absolute localization then at least at what may be termed vernier localization

The same conclusion arises from the data at 60 the data for Ss JDH and EBB may be compared with their data at 0 azimuth in Table I while the data for Ss. RLS and RLM may be compared with their data at 0° azimuth in Table II. If there is a trend for any S to perform better at 0 it is negligible in absolute amount

It seems likely that the striking deterioration of Mills Ss at 60 azimuth arose because a single sound source was moved physically from one position to another during the interval of silence between the two tones, and E was unable in this situation to measure and allow for a spatial constant error This conclusion accords with our common experience as we stand in a meadow and listen with eyes closed to an airplane moving across our right front quadrant we do not lose the distinctness of our impression of its direction as it moves from 0 and approaches 30 azimuth nor lose the impression altogether of where it is as it goes through the 60 azimuth

We therefore conclude that when the proper conditions are met, the human mechanism is capable of m a a. by this technique of the order of 1–2 at any azimuth and any frequency and with only a brief (1/4-sec or less) observation of the stimulus.

Exper V M A A with a Moving Stimulus

It occurred to us that an even better m a a. might be achieved if the sound source were not static, but continuously moving. In this case during the time of auditory observation S could continuously check and recheck his impression and as it were computerize a decision whether a sound source was moving L or R off the midline using as data the progressive interaural shifts in DI Dφ and or Dt as these were available

APPARATUS AND PROCEDURE

The DC motor for the upper track was arranged to move the loudspeaker at 2.5 /sec. As the speaker passed the midline moving either L or R, it tripped a microswitch which initiated the pure tone The azimuth over which the moving speaker was energized was controlled with a timer accurate to 1 msec A low level white noise masked any possible auditory cue to movement other than the pure tone S was blindfolded as usual and asked to judge whether the sound source moved L or R during the tonal interval

RESULTS AND DISCUSSION

Our first look at this problem was curtailed as it became apparent that far from this

ference between channels, seemed to come from about the 60 azimuth. This channel difference varied but was commonly about ±5 dB. We did not think it necessary to document this datum other than to have *S* indicate when the lateralization of the within-the head sensation seemed to be at about the same simulated azimuth as that of an actual sound source at 60 off midline. At this setting, *S* judged whether the second tone was L or R of the new phenomenological zero. Threshold DI was determined graphically as the 75% correct point.

Table VII. *Successive interaural intensive discrimination for pure tones*

Note: Stimuli tape-recorded and presented by earphones

Subjects	Frequency in KHz 0.5	1	1 6	3.2	6.4
0° Azimuth					
RLS	0.4	0.6	0.7	0.65	0.85
RLM	0.6	1.0	0.6	0.5	1.05
JDH	1.2	0.5	0.95	0.7	1.05
EBB	2.5	1.9	0.8	0.8	1.25
Mean	1.12	1 1	0.76	0.67	1.05
60° Azimuth					
RLS		0.5	0.9	0.5	
RLM		0.75	0.85	0.5	
JDH		1 1	0.9	1.1	
EBB		1 4	1.3	0.7	
Mean		0.96	0.99	0.7	

RESULTS AND DISCUSSION

Table VII reveals that the threshold interaural DI at a simulated azimuth of 0 is usually of the order of 1 dB or less, and that changing the simulated azimuth to 60 has no deleterious effect whatever Evidently the deterioration of some of the m.a.a. data at 60° azimuth is no consequence of a systematic bias of stimulus intensity in a dichotic situation where interaural intensity is asymmetric.

Individual data show no trend across frequencies, and we conclude that the ears are as well adapted to utilize interaural DI information at low frequencies (where it does not exist in the usual free field situation below about 1 kHz) as at 6.4 kHz, where physical interaural DI may far exceed the amount needed to lateralize and localize to the extreme L or R.

Exper VIII The Effects of the Human Pinnae on the M A A at 0° Azimuth and 0° Elevation

The general nature of the effect of the whole head on the physical cues for interaural DI as a sound source is swept around the horizon has been clear for decades (see, e.g. Sivian & White 1933) We were interested in the relatively minor effects contributed by the pinna, nose cheekbone, etc. in providing a particular individual with DI cues of a minimal sort upon which m.a.a. judgments of the order of 1 could be based. We found, in fact, that these irregularities on the surface f the head do have or could possibly have significance in the real world for any *S*.

APPARATUS AND PROCEDURE

With one *S* an attempt was made to remove the pinna from utility by taping it closely to the head, and smearing cold cream liberally leaving only a slightly tapering funnel leading to the canal proper The same apparatus was used as in former experiments for the acoustic zero, visual zero and moving-loudspeaker m.a.a. always with a 3-sec observation interval. Unfortunately time was available only for observations at the 0 azimuth, In all probability other azimuths and elevations would have been more revealing.

more efficient than with an acoustic zero Tables I vs. VI reveal that visual zero was better in 20 of 28 possible comparisons of individual data at the seven common frequencies. The mean trends are seen in Fig. 1

Whether the visual zero task would have been more efficient than the acoustic zero had the visual task been limited to a 1/4-sec observation as in Table I is an unanswered question at the moment. Certainly 3 sec gave *S* plenty of time to oscillate the head through several cycles but from the slight, if any advantage of head movements seen in Exper III we would predict that the visual-zero task could be performed quite well with only a 1/4-sec observation interval.

It is obvious to us that the eyes and ears are coordinated down to within a fraction of a degree azimuth, and that the quickest and most efficient way to collect m a.a. data is to have *S* read off azimuth of a hidden sound source from a graduated arc, placed at about the same distance as the sound source.

It is noteworthy that for three of the four *Ss*, performance at 1 6 kHz, while still good, was worse than at any other frequency

Exper VII Interaural Differential Intensive Sensitivity

A broad look at how it is possible for m.a.a. as small as 1 or less to exist would include studies of (1) the physical cues available and (2) efficiency of human performance in utilizing these cues one at a time and in concert Among the questions in the latter category for which we felt more information was needed was that of interaural intensive discrimination (DI) Some data by Mills (1960) indicated that threshold interaural DI was just about the same in dB as the physical interaural intensity differences available to *S* when a sound source is 1–2 off midline thus the latter could serve as a localization cue in a free field However we wanted to know in addition whether some of the differences in m.a.a among our *Ss* could be explained by differences in their interaural DIs, and we further wished to know how the DI behaved when the phantom sound used as the basis for L–R judgments was placed not at simulated 0 azimuth, but displaced a simulated 60 off midcenter

APPARATUS AND PROCEDURE

A two-channel tape was prepared on an Ampex PR 10-2C recorder consisting of pairs of tones (rise–fall times of 10 msec) each 1/2 sec long separated by 0 1 sec silence. The first of each pair was always equal in intensity on the two channels, giving the sensation of a tone burst in the middle of the head when played diotically to a normal hearing *S* wearing ear phones. In case of some slight asymmetry of loudness with equal intensity *S* adjusted the gain of Channel II until midline localization for the first tone was achieved.

The second of each pair of tones was recorded on Channel I at one of a selection of intensity differences from that of the first pair either ±0 0 1 0 2 0 3 0 4 0 5 0 6 0 8 1 0 1 3 or 1.5 dB and of the opposite sign and the same intensity differences on Channel II Thus, dichotic differences of ±0 0 2, 0 4 0 6 0 8 1 0 1 2 1 6 2.0 2.6 or 3 0 dB were accomplished for the second tone. *S* simply had to judge at 40 dB sensation level whether the second of each pair was phenomenologically L or R of the first. Ear and frequency order were of course counterbalanced.

In a second procedure the gains in dB of Channels I and II were changed respectively up or down for each *S* until he reported that the "0 items, i.e. those with no built in dif

ference between channels, seemed to come from about the 60 azimuth. This channel difference varied but was commonly about ±5 dB. We did not think it necessary to document this datum other than to have *S* indicate when the lateralization of the within-the head sensation seemed to be at about the same simulated azimuth as that of an actual sound source at 60 off midline. At this setting, *S* judged whether the second tone was L or R of the new phenomenological zero Threshold DI was determined graphically as the 75% correct point.

RESULTS AND DISCUSSION

Table VII reveals that the threshold interaural DI at a simulated azimuth of 0 is usually of the order of 1 dB or less, and that changing the simulated azimuth to 60 has no deleterious effect whatever Evidently the deterioration of some of the m.a.a. data at 60 azimuth is no consequence of a systematic bias of stimulus intensity in a dichotic situation where interaural intensity is asymmetric.

Individual data show no trend across frequencies, and we conclude that the ears are as well adapted to utilize interaural DI information at low frequencies (where it does not exist in the usual free field situation below about 1 kHz) as at 6.4 kHz, where physical interaural DI may far exceed the amount needed to lateralize and localize to the extreme L or R.

Table VII. *Successive interaural intensive discrimination for pure tones*

Note: Stimuli tape-recorded and presented by earphones

Subjects	Frequency in KHz 0.5	1	1.6	3.2	6.4
0° Azimuth					
RLS	0.4	0.6	0.7	0.65	0.85
RLM	0.6	1.0	0.6	0.5	1.05
JDH	1.2	0.5	0.95	0.7	1.05
EBB	2.5	1.9	0.8	0.8	1.25
Mean	1 12	1 1	0.76	0.67	1.05
60° Azimuth					
RLS		0.5	0.9	0.5	
RLM		0.75	0.85	0.5	
JDH		1 1	0.9	1 1	
EBB		1 4	1.3	0.7	
Mean		0.96	0.99	0.7	

Exper VIII The Effects of the Human Pinnae on the M.A A at 0° Azimuth and 0° Elevation

The general nature of the effect of the whole head on the physical cues for interaural DI as a sound source is swept around the horizon has been clear for decades (see, e g. Sivian & White, 1933) We were interested in the relatively minor effects contributed by the pinna, nose, cheekbone, etc., in providing a particular individual with DI cues of a minimal sort upon which m.a.a. judgments of the order of 1–2 could be based. We found, in fact, that these irregularities on the surface of the head do ha e or could possibly have significance in the real world for any *S*.

APPARATUS AND PROCEDURE

With one *S* an attempt was made to remove the pinna from utility by taping it closely to the head, and smearing cold cream liberally leaving only a slightly tapering funnel leading to the canal proper The same apparatus was used as in former experiments for the acoustic zero, visual zero and moving-loudspeaker m.a.a., always with a 3-sec observation interval. Unfortunately time was available only for observations at the 0 azimuth in all probability other azimuths and elevations would have been more revealing.

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JDH	1.2	0.5	0.95	0.7	1.05
EBB	2.5	1.9	0.8	0.8	1.25
Mean	1.12	1.1	0.76	0.67	1.05
60° Azimuth					
RLS		0.5	0.9	0.5	
RLM		0.75	0.85	0.5	
JDH		1.1	0.9	1.1	
EBB		1.4	1.3	0.7	
Mean		0.96	0.99	0.7	

RESULTS AND DISCUSSION

Table VII reveals that the threshold interaural DI at a simulated azimuth of 0° is usually of the order of 1 dB or less, and that changing the simulated azimuth to 60° has no deleterious effect whatever. Evidently the deterioration of some of the m.a.a. data at 60° azimuth is no consequence of a systematic bias of stimulus intensity in a dichotic situation where interaural intensity is asymmetric.

Individual data show no trend across frequencies, and we conclude that the ears are as well adapted to utilize interaural DI information at low frequencies (where it does not exist in the usual free field situation below about 1 kHz) as at 6.4 kHz, where physical interaural DI may far exceed the amount needed to lateralize and localize to the extreme L or R.

Exper. VIII. The Effects of the Human Pinnae on the M.A.A. at 0° Azimuth and 0° Elevation

The general nature of the effect of the whole head on the physical cues for interaural DI as a sound source is swept around the horizon has been clear for decades (see, e.g. Sivian & White, 1933). We were interested in the relatively minor effects contributed by the pinna, nose cheekbone etc. in providing a particular individual with DI cues of a minimal sort upon which m.a.a. judgments of the order of 1–2° could be based. We found, in fact, that these irregularities on the surface of the head do have or could possibly have significance in the real world for any S.

APPARATUS AND PROCEDURE

With one S an attempt was made to remove the pinna from utility by taping it closely to the head, and smearing cold cream liberally, leaving only a slightly tapering funnel leading to the canal proper. The same apparatus was used as in former experiments for the acoustic zero, visual zero, and moving-loudspeaker m.a.a., always with a 3-sec observation interval. Unfortunately time was available only for observations at the 0° azimuth in all probability other azimuths and elevations would have been more revealing.

Table VIII *Minimum audible angle at 0° azimuth by three methods with and without obliterating the features of the pinna with cold cream*

S EBB. Stimulus duration 3 sec

Frequency in KHz	Acoustic zero		Moving sound		Visual zero	
	Without	With	Without	With	Without	With
0.125	2.4	2.05	1 7	3.3	[illegible]	2.15
0.2	2.0	2.15	1 6	1 8	1 1	0.9
0.2	0 9	0.8	0 9	0.8	1 0	1 5
0.8	0 4	1.2	0 7	0.6	1 0	3 0
1.25	1 7	1 05	1 0	2.15	1.3	1 6
1 6	0.7	1 4	1.5	1 4	1 6	4 1
3.2	1 4	0.4	1 6	[illegible]	1 7	1 8
6.4	2.3	0 8	0.9	1.3	0.9	1 4

RESULTS AND DISCUSSION

Table VIII (see also Fig 3) reveals that obliteration of the distinctive features of the pinna had for this *S* no effect on m a.a. either for the acoustic zero task or the moving speaker task. There does, however seem to have been some contribution of the pinna to m a.a. at 0 8 and 1 6 kHz for the visual zero task Recall that with such a task (see again Table VI) this S did not show a relative break down at 1 6 kHz, although other *Ss* did. Now we see that even with this *S* when the pinna is reduced in effectiveness, m a.a. at 1 6 kHz is especially elevated as also its subharmonic 0 8 kHz. What this may tell us about the possible relation between wavelength at 1 6 kHz (about 8 inches) and the individual heads of our *Ss* will depend on what we can find out about individual differences of various sorts in this population At least we can gather from Table VIII that this S can use her pinnae to reduce certain ambiguities in L–R directionality when the half wavelength is of the order of the interaural distance.

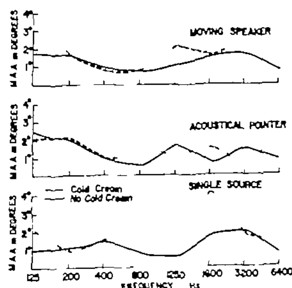


Fig 3 Minimum audible angle as a function of frequency at 0° azimuth by three procedures, each with or without the contribution of the pinnae. *S* EBB

Exper IX The Effects of the Sound Shadow of the Artificial Head and of its Pinnae Separately

Jahn & Vogelsang (1959) and Schirmer (1963) with real human heads, and Nordstrom (1962) with an artificial head among others, have recently made most detailed surveys of interaural physical DI as either azimuth and/or elevation is changed. These sets of data are for the case of the whole head including pinnae With the artificial head in Fig. 2 we were

able to document the sound shadow thrown by the whole head (1) including either of two sizes of pinna, or (2) with no pinnae at all. Obviously one can determine (3) the physical effects of the pinnae by subtracting (2) from (1).

APPARATUS AND PROCEDURE

The artificial head was mounted on a Bruel and Kjaer Model 3921 turntable, geared to a Model 2305 graphic level recorder fitted with polar coordinate paper and arranged so that the head looked directly at a triaxial speaker 8 ft distant. Either the head or the loudspeaker could be elevated so that the speaker was 30 above or below the level of the interaural axis. With this arrangement, two possibilities are offered. (1) the loudspeaker may be fixed at some relative azimuth and elevation and a recording from one eardrum taken with the frequency swept over 0 1–10 kHz, or (2) the frequency and elevation may be fixed and the head rotated so that the output from the eardrum represents the polar sound shadow at that frequency and elevation. We took the opportunity to do both.

The record for each frequency sweep, at any fixed geometry tells us what effect any particular frequency has on the soundshadow at that geometry as compared to the standard geometry of 0 azimuth and 0 elevation. Unfortunately we did not then have a vailable compensatory circuitry to present constant SPL at all frequencies to the head thus these data must first be corrected for the frequency response of the loudspeaker

It seemed to us that such information through the usable audio range could be abstracted by picking off the data for a selection of discrete frequencies from 0.5–8 kHz.

RESULTS AND DISCUSSION

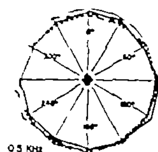
Fig. 4 A–C shows the data for four frequencies at each of the elevations (+30, 0, and −30) and at each of the 12 30 -step azimuths.

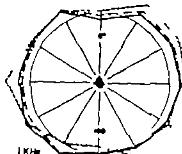
The points on these polar plots are not, however direct sound shadows. Each point represents a *difference* in intensity at the two ears, with the further conventions (1) that every datum to the R of the 0–180 meridian represents the case for the R ear and to the L for the L ear and (2) that the perfect circle drawn at an arbitrarily chosen radius represents the case where the SPLs at the two ear drums are equal. Thus in Fig. 4 B at 8 kHz, for the case of the head without pinnae (dotted line) at 60 azimuth, the interpretation is that for that particular frequency and geometry the R ear yields 11 dB more SPL than the L, while at −60 azimuth (+300) the L ear yields 12 dB more SPL than the R. Note carefully again that each point does not show the sound shadow cast at one ear alone, as in the usual display of such data, but the algebraic summation of the two shadows, one at each ear

The effects of pinnae of two sizes, can also be abstracted from Fig. 4 If we redraw the interaural DIs for the cases of the large and the small pinnae, each in terms of what they add or subtract from the interaural DI for the case of the head without pinnae, the effects of the latter are clear Fig. 5 A–C shows such data, where the perfect circle drawn at the arbitrarily chosen radius represents not interaural DI of 0, as in Fig. 4 but whatever the interaural DI was for the head with no pinnae at that azimuth and elevation of interest. Thus in Fig. 5 B at 8 kHz, for the case of the head fitted with the large pinnae at 60 azimuth, the interpretation is that for that particular frequency and geometry these pinnae reduce the interaural DI by 7 dB

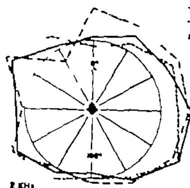
One is struck, indeed, by the extent to which the pinnae of both sizes act rather to *reduce* the DI thrown by the head alone for all three elevations, twelve azimuths, and four frequencies represented, the pinnae reduce the interaural DI on 184 occasions out of 288, whereas the expectation would ordinarily be

ELEVATION





NO
SMALL
LARGE
5 dB



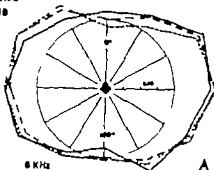
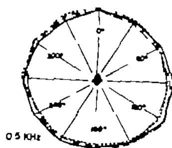
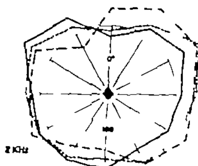


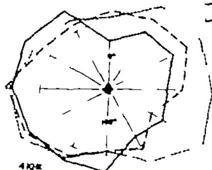
Fig. 4. A–C. Sound shadows thrown at representative frequencies and three elevations by a loudspeaker rotating around the artificial head without pinnae and with either large or small pinnae. Note, the perfect circle represents symmetry; a point further from the center indicates the sound was more intense in the ear on that side of the midline.

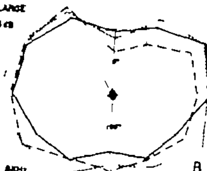
0 ELEVATION





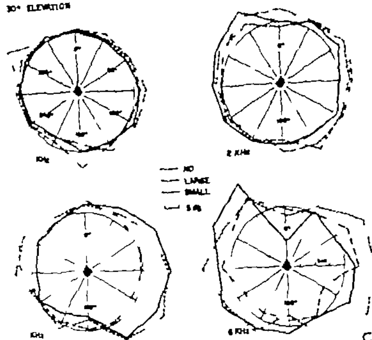
NO
SMALL
LARGE
5 dB





that the pinnae should act to *increase* the interaural DI cue and thus promote directionality. Considering the cases in Fig. 4 A–C, where the head alone throws quite different sound shadows at fairly close azimuths, it may be that the pinnae act to smooth out in some degree these saltatory conditions.

Figs. 4 and 5 show that for every head the



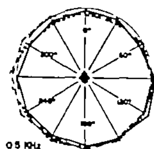
effect on interaural DI is unique for every frequency and geometry. One effect of this must be that for each of us the timbre of sounds will change with azimuth and/or elevation. Fig. 6 shows this graphically. Consider a white noise, with all frequencies of equal physical intensity yielding a certain sensation when the source is at 0° azimuth and 0° elevation. On the graphs of Fig. 6 this condition is presented by a horizontal line at 0 dB intensity at all frequencies. If now the sound source is changed to for example 60° azimuth and 30° elevation, Fig. 6 tells us that the timbre of the noise has a broad high-frequency boost for the ear nearer the sound source at all frequencies of 1–2 kHz and above, for the head either with or without the pinnae. And a comparison of curves for the 18 geometries in Fig. 6 A–C convinces one that the timbres of the white noise or of any complex sound will be quite different for each point in space, and for each set of pinnae. Thus through the intermediary of the sound shadow one could learn to associate the timbre of familiar sounds with their relative location.

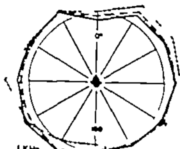
Exper. X. Sound Shadow of the Human Head at ±5° Azimuth

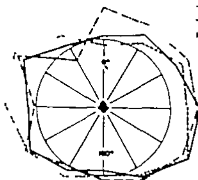
APPARATUS AND PROCEDURE

With both meati fitted with small vaseline cotton plugs (to avoid canal resonances) and with probe microphones ending at the plane of the cavum concha, swept-frequency recordings were taken at both ears (S. JDH) of SPL with the sound source at 355°, 0° and 5° azimuth. A bite board helped stabilize geometry.

30 ELEVATION







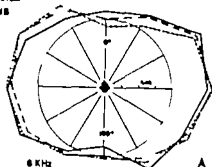
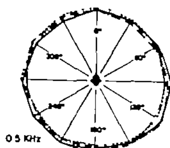
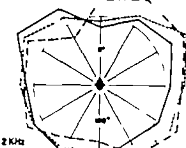
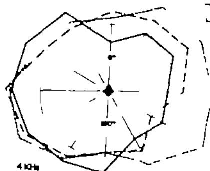


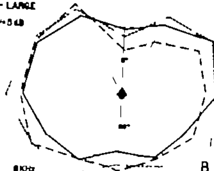
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0 ELEVATION



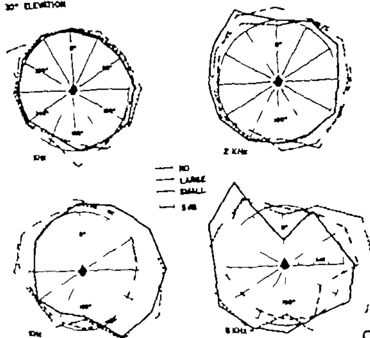






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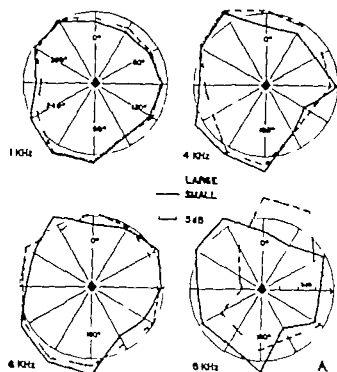


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Exper. X. Sound Shadow of the Human Head at ±5° Azimuth

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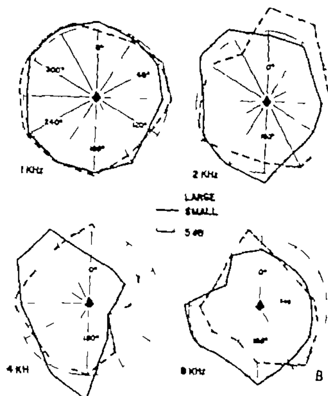


Fig 5 A–C. Abstracted from Fig. 4 A–C The effect of the pinnae alone on the sound shadow Note The perfect circle here represents the sound shadow if any thrown by the head alone

RESULTS AND DISCUSSION

Fig. 7 shows that at only 5 off midline there were interaural DIs in extent quite sufficient at most frequencies to support good localization on the basis of intensity alone.

Exper XI Minimum Audible Field at ±5 Azimuth

APPARATUS AND PROCEDURE

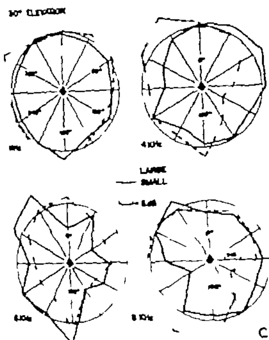
A closer look at the usefulness of physical DI cues in this azimuth region was had by asking *Ss* to take detailed monaural audiometric threshold tracings as the sound was swept from −5 to +5 azimuth A speed of 0 5 min/degree was arranged a frequency chosen (1 6 3 2 6 4 or 10 kHz) and *S* (with L ear plugged) traced his R ear Bekesy threshold as he sat blindfolded with a bite board facing the 0 azimuth. Control observations were also made at 30 60 and 90 azimuth.

On our Reger Bekesy audiometer not only may one vary the speed of frequency sweep, but also attenuation speed this was sometimes adjusted to that which gave the best indication of trends in available dB/degree

To satisfy ourselves that the technique gave reliable data for each *S* three tracings were collected for each frequency at each of the four main azimuths 0–90 a total of 48 tracings per *S*

RESULTS AND DISCUSSION

Fig 8 A–D shows representative data Fig. 8 C illustrates the test-retest consistency at 1 kHz with one *S* and 8 D at 6.4 kHz in another *S* Very commonly there was an indi



cation in the tracing itself of exactly when the sound source crossed the 0 azimuth, and in almost every tracing there are repeatable differences between the azimuths in the 10 range which amounted to 2+ dB/degree, that is, quite sufficient to account for discriminable azimuths a very few degrees apart. Thus physical cues for loudness would be enough to subserve the m.a.a. found in Fig. 1

These observations apply especially to the region of the midline· but even at other azimuths there were differences across a 10° range which could subserve usable m.a.a.

Exper XII M A A with Interaural DI as the Only Cue

In all experiments thus far in the free field, all possible physical cues were always present, so that it is impossible to decide which cues may be prepotent for or even used at all by the auditory mechanism Experimental ways were devised to isolate the cues of intensity and time separately The cue of phase could not be isolated in the free field in an unadmixed fashion, but it could at least be pre ented from obtruding by the choice of a fre quency 3 2 kHz, far above that known to be important for DØ under earphones. This would lea e DI and Dt as possible physical cues We looked first at DI in isolation.

APPARATUS AND PROCEDURE

S using a bite board was placed as usual 10 ft perpendicular from the center of the alu the loudspeaker could be moved and positioned L–R as needed. Behind the opaque screen the usual L–R placements were counterbalanced to determine m.a.a. at the 75%-correct angle. However S never heard the real initiation or termination of the pure tones. Directly behind him a few feet away was a second loudspeaker energized as needed by a white noise of about 80 dB SL, i.e., more than enough to mask any possible sound from the test speaker in front. Keying of the speakers was timed so that S first heard the white noise, after which the front speaker (positioned either L or R but quite inaudible) was energized, then after 1/2 sec the noise was turned off for either 1/4 sec or 5 sec, then on again, and after another 1/2 sec the tone (now again inaudible) was terminated, after which the noise also was turned off and the trial terminated with S's judgment L–R of the pure tone.

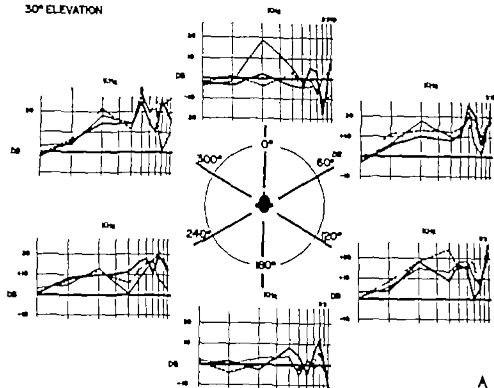
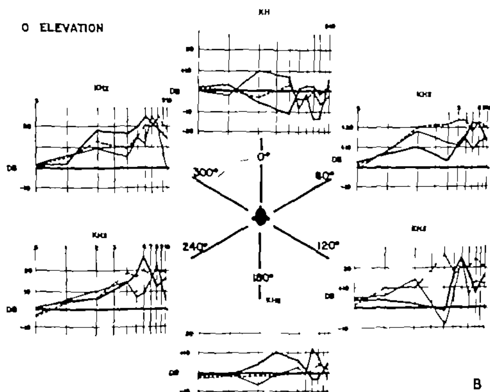
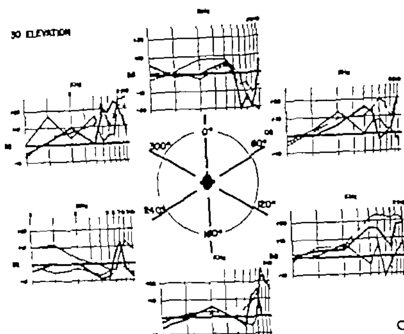


Fig 6 A–C. Effect of the sound shadow on timbre as a function of azimuth. Note. Graphs to the R L of the midline represent the increase in SPL at the R ear over the L, or L over R respectively



By this method of placing the masking loudspeaker in the midline the Dt cues on initiating and terminating the tone were removed from *S* and he was given a temporally non informative window during which he could judge the pure tone L–R Of course



control data were also taken with the 5-sec tone with no masking, i.e. with both DI and Dt information.

RESULTS AND DISCUSSION

Table IX shows no trend for m.a.a. to worsen upon removal of the timing information, even with only a 1/4-sec observation window

A first conclusion might be that timing information is unimportant in localization, but this would be absurd from everyday as well as from laboratory observations. The DI cues as a sound source sweeps around the head as, for example, one stands in a free field and listens to a sound changing azimuth, do not give the impression of sudden shifts of position, as would be the case if the almost erratic DI cues at many frequencies (as seen in Figs. 4 5) were the sole source of directionality Furthermore, experiments too numerous to mention tell us that we are in fact exquisitely sensitive to Dt even in the absence of DI cues.

The case must be that this Exper XII tells us not that DI is prepotent over other cues, but that the auditory mechanism can seize upon any cue available, and make use of it in an efficient manner

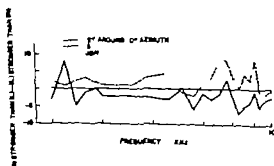


Fig. 7 Effect of the sound shadow at ±5 azimuth on interaural physical DI. S. JDH.

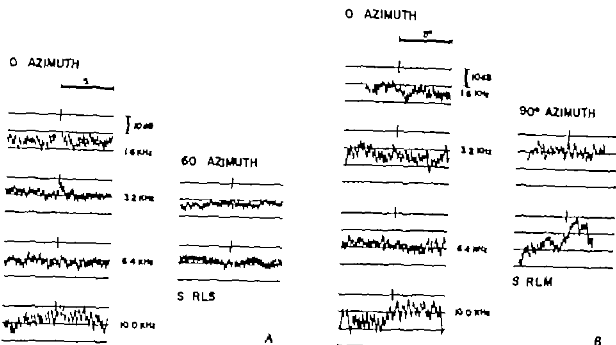


Fig 8 A D Effect of sound shadow on *monaural* minimum audible field across ± 5 azimuth

Exper XIII M A A with Interaural Dt as the Only Cue

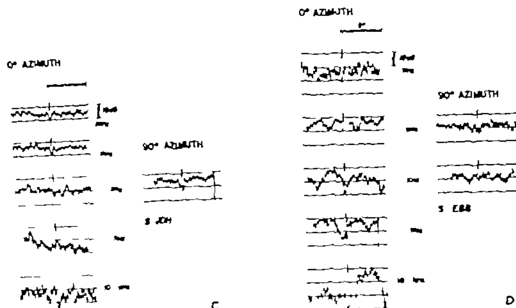
The converse of Exper XII was arranged, in which an attempt was made to eliminate DI cues while retaining and even exaggerating Dt cues. This experiment was performed by Steinbach (1965) under somewhat different auspices, and under the more direct guidance of Dr Jack Curtis.

APPARATUS AND PROCEDURE

The larger of two sets of pinnae from the Shilling artificial head were mounted on the ends of semi soft plastic tubes, i d = 3/8 inch o d. = 1/2 inch and the other ends sealed into the external auditory meati of 3 adult *Ss* in turn The attenuation of the tubes was 10 dB for a band of noise 220–780 Hz, and 25–29 dB for the bandwidth 740–4 000 Hz. The helmet and tubes allowed for variable tube lenghts to the two ears when the array was supported by a plastic helmet worn by *S* These lengths were either the same or of somewhat different lengths, but always with the same inter pinna distance of 9 5 inches. It could be shown for the case of a sound source off the midline that the interaural Dt per degree azimuth available for L–R judgments was about 10 6 μsec/degree azimuth for the head with no pinnae about 13 3 μsec/degree for the head with inter pinna distance of 9 5 inches and equal-length tubes and about 19 μsec/degree for the same inter pinna distance but with tubes of unequal

Table IX *Contribution to M A A at 3 ? KH (visual ero") possible on interaural intensity cues alone*

Subjects	Tone on 5 sec With timing information	Tone on 5 sec Without timing information	Tone on / s Without timing information
RLS	0.5	1	1 2
RLM	4 1	4.0	1 9
JDH	5	3 3	3 9
EBB	1 5	0.9	2
Mean	1 5	35	8



length as used here. Thus the condition of unequal-length tubes gives an additional temporal cue of 19–13.3 = 5.7 µsec/degree azimuth re the equal-length condition, and if Dt of this order of magnitude can be used as a cue, m.a.a. should be somewhat superior in the condition of inequality. The fact that DI with this apparatus is negligible was shown by placing the Shilling artificial head, fitted with this apparatus, in the anechoic chamber at the Medical Research Laboratory and recording the sound shadow around the head. A loudspeaker was energized with the bands of noise separately and the output plotted of a Western Electric 640AA microphone at the position of the eardrum. Steinbach's Fig. 14 shows that for the band 740–4 000 Hz, and of course at all lower ranges, there was less than a 1-dB variation across any ±5° azimuth change, over the octant from 0–45°. Thus in this experiment Ss had only Dt to guide them, not DI.

M.a.a. were collected by a method modelled on Mills (1958) on all 3 Ss at 0, 14, 30, and 45° for a low, a middle, and a high frequency noise band without the helmet, and with the helmet bearing equal and unequal tubes.

RESULTS AND DISCUSSION

When results were converted from m.a.a. to Weber fractions so that data from all 4 azimuths could be collapsed, Steinbach's Figs. 11–13 show that all Ss exhibited lawful curves for the two lower bandwidths mentioned. Data from a higher bandwidth of 2.1–5.9 kHz were erratic for all Ss, but are in any case for this purpose uninterpretable since the attenuation difference between the two unequal-length tubes was about 3 dB, and the sound shadows thrown amounted at some azimuths to as much as 0.55 dB/degree (i.e., enough to yield an m.a.a. of 1–2° on a DI cue alone).

These data are interpreted to mean that all Ss used Dt alone to yield quite good m.a.a. In Steinbach's Figs. 11–13 it can be seen that the Dt data from the helmet usually at least equal, and for one S often exceed, control m.a.a. with no helmet, in which of course all possible cues are present.

Whether the increased cue in µsec/degree in the unequal-tube condition, as compared with the equal-tube condition, is made use of by any S is unclear in Steinbach's data.

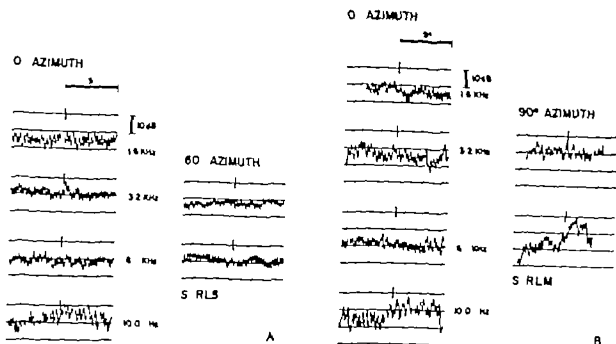


Fig. 8 A D Effect of sound shadow on monaural minimum audible field across ±5 azimuth

Exper XIII M A A with Interaural Dt as the Only Cue

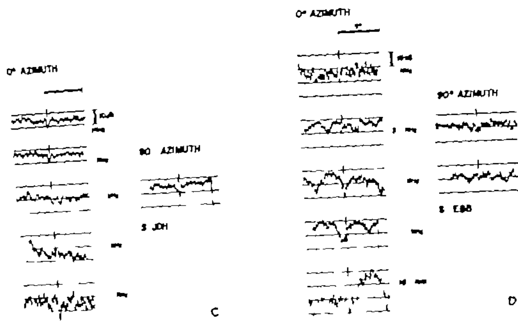
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Table IX *Contribution to M A A at 3.2 KH ("visual zero) possible on interaural intensity cues alone*

Subjects	Tone on 5 sec: With timing information	Tone on 5 sec: Without timing information	Tone on / s: Without timing information
RLS	0.5	1	1
RLM	4 1	4 0	3 9
JDH	2.5	3 3	3 9
EBB	1 5	0 9	—
Mean	_15	35	8



length as used here. Thus the condition of unequal-length tubes gives an additional temporal cue of 19–13 3 = 5.7 μsec/degree azimuth re the equal-length condition, and if Dt of this order of magnitude can be used as a cue, m.a.a. should be somewhat superior in the condition of inequality The fact that DI with this apparatus is negligible was shown by placing the Shilling artificial head, fitted with this apparatus, in the anechoic chamber at the Medical Research Laboratory and recording the sound shadow around the head. A loudspeaker was energized with the bands of noise separately and the output plotted of a Western Electric 640AA microphone at the position of the eardrum Steinbach s Fig 14 shows that for the band 740–4 000 Hz, and of course at all lower ranges, there was less than a 1-dB variation across any ±5 azimuth change, over the octant from 0–45 Thus in this experiment Ss had only Dt to guide them, not DI

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RESULTS AND DISCUSSION

When results were converted from m.a.a. to Weber fractions so that data from all 4 azimuths could be collapsed, Steinbach s Figs. 11–13 show that all Ss exhibited lawful curves for the two lower bandwidths mentioned for the two lower bandwidth of ∠.1–5 9 kH Data from a higher bandwidth of ∠.1–5 9 kH were erratic for all Ss, but are in any ca for this purpose uninterpretable since the at tenuation difference between the two un equal-length tubes was about 3 dB and th sound shadows thrown amounted at some az muths to as much as 0.55 dB/degree (Le enough to yield an m.a a. of 1–2 on a D cue alone).

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Although these data were not collected in an echo-free chamber but in an I.A.C. booth 8×10×6 5 ft at General Dynamics/Electric Boat Co Groton Conn. it is not likely that secondary reflections rendered invalid all con clusions.

A more precise comparison between the equal- vs. the unequal-tube condition with much more sharply filtered and restricted bandwidths, and control of DI cues, would be desirable to bear on the questions (1) whether Dt information is as useful at the higher fre quencies, as we would predict and (?) whether the auditory system can yield better m.a.a. than normal when provided with the auditory analogue of a visual range finder

Exper XIV M A A with Noncongruent Physical Cues Symmetrical Dt but Asymmetrical DI

What would be needed to establish prepotency of any cue would be a situation in which two and only two cues were present, but direc tionality of opposite meaning We were able to generate a situation in the free field which *is one analogue of* the *common* DI–Dt *trad* ing *experiments* under headphones, where the Dt cue is as if the sound source were to the L *of midline*, the DI cue to the R. The con tribution of several parameters to the trading relation (in dB/μsec) under headphones is well known. It is unknown in the free field.

APPARATUS AND PROCEDURE

The visual "zero problem was set up as usual and m.a.a collected with a 1/4-sec and a 5-sec "look" at the stimulus, but on random occa*sions half* of the 5-sec trials were performed with a cotton plug in one ear canal. The ef *fect of this plug* at *3.2 kHz* was ascertained by collecting Bekesy tracings in a free field before, *during* and after inserting the cotton. The attenuation provided (6–8 dB see Table X) would be enough for complete lateraliza tion were these interaural DI conditions created under earphones (see Exper VII above)

Table X *Effect on minimum audible angle at 3.2 KH ("visual zero") of noncongruous intensity and timing cues*

	Tone on 5 sec		Tone on 1/4 sec	
Subject	Both ears open	R ear plugged	Both ears open	Attenuation of plug in Db
RLS	3 6	3 9	3 6	8
RLM	*0.9*	*0.6*	*1 1*	6
JDH	3.3	.6	3.9	7
EBB	*1.0*	1 4	1.9	6
Mean	2.2	2.1	.6	7

RESULTS AND DISCUSSION

Although the 6–8 dB DI would be enough by itself to displace the sound image drastically under headphones, it was not enough to counteract very strongly the prepotent Dt cues *Ss* reported that the image when at known 0 azimuth was seemingly displaced a few degree toward the open ear but once *this fact was accounted for* Table X shows that for no S were the m.a.a. worse when a pronounced asymmetry in intensity was introduced. Ordinary experience of course clearly shows that drastic changes in DI do not entirely dislocate sound images, as one can at test who puts his finger repeatedly in his ear *at a cocktail party* and certainly a trading relation exists at the larger DIs but within the region of DI asymmetry of at least 6–8

dB we could establish that Dt takes precedence almost to the exclusion of DI cues.

This experiment of course accords with common experience—those who have upper respiratory infections with reduced audiometry more in one ear than the other for a few days do not feed one cat when its fellow meows, or have any difficulty navigating in traffic. It is possible that very rapid relearning occurs when DI is asymmetrical in ordinary life, but relearning was not apparent in the data from Table X, and it seems therefore an unlikely necessity in daily life.

We conclude as a working hypothesis that Dt cues are used for the most part in localization of sounds in space, and take precedence where other incongruous cues exist, but that if the organism is thrown back upon DI cues, these too can subserve good performance.

Summary

Fourteen experiments were performed on directional hearing in humans in an anechoic chamber of 37 328 cubic feet. Minimum audible angles (m.a.a.) were determined from 125 through 6 400 Hz with four very experienced adults. Sets of m.a.a. were provided by the Method of Constants using either an acoustic or visual cue to signalize zero azimuth, and for the case of a continuously-moving sound source. The acoustic and visual zero experiments yielded m.a.a. of 1–2° at all frequencies, with either a 1/4-sec or 3-sec observation of the stimuli and (once a constant error due to geometry was controlled) at 30 and 60° azimuths. M.a.a. for the moving source yielded somewhat coarser m.a.a. probably due to the fact that both source and head were moving simultaneously. The effect of head movements on the acoustic and visual zero experiments was negligible at 0° azimuth. Successive interaural differential intensity discrimination was equally good at all frequencies and phenomenological azimuths. When the m.a.a. in the free field with cues both of interaural time-of-arrival and of interaural intensity were compared with control studies in the free field eliminating one or other of these cues, and when the two cues were rendered non-congruent, it was found that the m.a.a. by either cue alone was enough to support good m.a.a. at all frequencies; but when the two cues were rendered non-congruent by special techniques in the free field, the cue of time-of-arrival (and of termination) were propotent over the intensity cue up to at least 6–8 dB interaural intensity. Thus a rather large extent of interaural intensity difference exists over which the expected DI/DT trading did not develop, emphasizing the relatively profound effect which time relationships have on directionality.

Zusammenfassung

Vierzehn Experimente wurden in einem echototen Raum von 37 328 Quadratfuss, am Richtungsgehör in Menschen gemacht. Minimumhorbare Winkel (m.h.w. auf englisch m.a.a.) wurden von 125 bis 6 400 Hz mit vier sehr erfahrenen Erwachsenen errechnet. Die Sammlung von m.h.w. wurden durch die Methode der Konstanten gefunden, durch Gebrauch eines akustischen oder visuellen Winkes, um Azimut-Null zu signalisieren, und im Falle einer sich stets bewegenden Schallquelle. Die akustischen und visuellen Null-Experimente ergaben m.h.w. von 1–2 Grad bei allen Frequenzen, entweder mit

Occasional readings from Ss Nos. 2 and 3 trend in that direction.

Although these data were not collected in an echo-free chamber but in an I.A.C. booth 8 × 10 × 6 5 ft at General Dynamics/Electric Boat Co Groton Conn it is not likely that secondary reflections rendered invalid all con clusions.

A more precise comparison between the equal- vs. the unequal tube condition, with much more sharply filtered and restricted bandwidths, and control of DI cues, would be desirable to bear on the questions (1) whether Dt information is as useful at the higher fre quencies, as we would predict, and (?) whether the auditory system can yield better m.a.a. than normal when provided with the auditory analogue of a visual range finder

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Table X. *Effect on minimum audible angle at 3 2 kH ("visual zero") of noncongruous intensity and timing cues*

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RLS	3.6	3.9	3 6	8
RLM	0.9	0 6	1 1	6
JDH	3 3	.6	3.9	7
EBB	1 0	1 4	1.9	6
Mean	2.2	.1	.6	7

RESULTS AND DISCUSSION

Although the 6–8 dB DI would be enough by itself to displace the sound image drastically under headphones, it was not enough to counteract very strongly the prepotent Dt cues. Ss reported that the image when at known 0 azimuth was seemingly displaced a few degree toward the open ear but once this fact was accounted for Table X shows that for no S were the m a.a. worse when a pronounced asymmetry in intensity was intro- duced. Ordinary experience of course clearly shows that drastic changes in DI do not en tirely dislocate sound images, as one can at test who puts his finger repeatedly in his ear at a cocktail party and certainly a trading relation exists at the larger DIs but within the region of DI asymmetry of at least 6–8

einer viertelsekundigen oder dreisekundigen Wahrnehmung der Erregungsfaktoren und (nachdem ein gewisser Fehler wegen Geome trie reguliert wurde) bei 30 Grad und 60 Grad Azimut. M h.w für die sich bewegende Quelle ergab etwas rohere m.h.w wahrscheinlich wegen der Tatsache dass sowohl Quelle wie Kopf sich gleichzeitig bewegten. Die Wirkung der Kopfbewegungen auf die akustischen und visuellen Null Experimente war unbedeutend bei Null Grad Azimut Die erfolgende inter aurale differentiale Intensitätsunterscheidung war bei allen Frequenzen ebensogut Als die m h w im freien Feld mit Winken von der interauralen Ankunftszeit und von der inter auralen Intensität mit der den einen oder den anderen Wink ausschliessenden kontrollierten Forschung im freien Feld verglichen wurden, und die beiden Winke nicht kongruent gemacht wurden, wurde entdeckt dass die m.h.w von einem der beiden Winken ge nügten, um gute m h.w bei allen Frequenzen zu unterstützen aber als die zwei Winke durch besondere Ausführungsarten im freien Feld nicht kongruent gemacht wurden, waren die Ankunftszeit und Endwinke vorherrschend über den Intensitätswink bis zu mindestens 6–8 dB interauraler Intensität. Daher existiert eine ziemlich grosse Ausdehnung von inter auralem Intensitätsunterschied, über den der erwartete DI/DT Handel sich nicht ent wickelte, was die ziemlich grosse Wirkung be tont die die Zeitverhältnisse auf Richtungsgehör haben.

Acknowledgment

Several institutions and individuals contributed to this series of experiments. Major support was provided to the C W Shilling Auditory Research Center Groton, Conn. through Contract RD-510 with the US Office of Vocational Rehabilitation and a coetaneous grant generously provided by The Seeing Eye Morristown New Jersey The use of workspace and aluminum loudspeaker tracks at the U.S.N Submarine Medical Research Laboratory Groton Conn. is gratefully acknowledged The Department of Speech at the University of Connecticut kindly supported the drawings of the figures and provided time for writing. Dr Jack Curtis kindly created the tapes used for studying minimum audible angle without head movement and tapes for interaural intensity discrimination. Thanks are also expressed to the subjects who contributed many hours over two years time to the judgments upon which this paper is based.

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SUPPLEMENT 299

Hyperosmosis of Endolymph as Primary Pathogenic Mechanism of Menière's Disease and Its Clinical Management

BY

Z. GODLOWSKI M.D.

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 299

Hyperomosis of Endolymph as Primary Pathogenic Mechanism of Menière's Disease and Its Clinical Management

BY

Z. GODLOWSKI, M.D

From the Copernicus Medical Research Foundation, Inc.
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Introduction

Menière's disease is a term used synonymously with endolymphatic hydrops, labyrinthine dropsy, labyrinthine hydrops, labyrinthine hypertension or even Menière's syndrome. According to Dorland's Medical Dictionary "disease" refers to a group of related symptoms due to a single pathological cause, whereas "syndrome" refers to a group of symptoms occurring together but of diverse causation. The term "Menière's syndrome" is used to denominate all forms of dizziness, vertigo or even ataxia, whether intermittent, constant or slowly remitting, and due to causes of diverse origin such as chronic suppurative otitis media, intracranial vascular accidents or even neurasthenia. A meaning so broad makes the term "Menière's syndrome" inadmissible in a scientific discussion. H. Williams (1965) therefore suggested that this term be discarded and the term "episodic vertigo" substituted in instances of periodic vertigos caused by diverse pathogenics of the labyrinthine system, and that Menière's disease be reserved to those instances of recurring intermittent vertigos meeting the criteria recommended by the Committee on Vertigo by the National Institute for the Study of Neurological Diseases and Blindness (1961).

The etiology of Menière's disease is not completely understood. It is generally accepted that the main pathogenic factor responsible for the clinical symptoms of labyrinthine hydrops is increased hydrostatic pressure within the endolymphatic system (Cawthorn, 1947; Shambaugh, Jr., 1959, 1966, 1968; House, 1964, 1968; Wittmack, 1929; H. Williams, 1965). However, the primary cause which leads to accumulation of endolymph is not uniform and probably varies from one case to another. Therefore, endolymphatic hypertension may have differing symptoms such as deafness, incapacitating vertigo, roaring tinnitus, diplacusis, etc. Manifestations of endolymphatic hypertension are commonly interrupted by a spontaneous remission. Such spontaneous fluctuations complicate the assessment of the efficiency of various therapeutic methods.

At the present time therapy of Menière's disease is restricted to symptomatic alleviation of the incapacitating manifestations by surgical and/or pharmacological decompression of the increased endolymphatic pressure.

The physiology of the inner ear has been the topic of much research with the hope that understanding of normal function of the inner ear would provide clues to understanding the pathological process responsible for Menière's disease. The increased endolymphatic pressure associated with Menière's disease must be viewed according to the anatomical characteristics of the inner ear and the physico-chemical and physiological principles controlling the formation and circulation of the endolymph. Throughout this paper, the more explanatory term "endolymphatic hypertension" will be used interchangeably with Menière's disease and endolymphatic hydrops. A proper plan of treatment will derive from an analysis of the etiology of the endolymphatic hypertension.

Presented at the Meeting of the Texas Ear, Nose, and Throat Society, May 7, 1971, in Houston, Texas.

Clinical manifestations of endolymphatic hypertension

Typical endolymphatic hydrops was shown by H. Williams (1965) to be a "cluster type" of

A primary or secondary disturbance in microcirculation may become a source of accumulation of lymph in any tissue, and this rule can be applied to the circulation of the endolymph. The diagram in Fig. 1 indicates four areas in which a disturbance might cause an *increase in volume of the lymph*, these four potential physiologic errors are therefore analyzed in an abbreviated form.

1 An elevation of the hydrostatic head pressure at the arterial end of the microcirculation (in case of Menière's disease in stria vascularis) *will increase the force which drives* fluid from the capillaries into extra-capillary space (in our case into the endolymphatic space). In such an event the hydrostatic pressure within the endolymph will rise only if the *excess fluid is not eliminated* at an equal rate *back into the blood* either at the venous end in the stria vascularis or by the excretory function of the endolymphatic sac, or by both.

2. Reduction of the osmotic pressure within the blood plasma in the capillaries (e.g. in *hypoproteinemia*) will reduce the return of fluid at the venous end of the capillary bed from the extracapillary space (in our case from the endolymphatic space). An accumulation of water within the endolymph would then occur even though the filtration at the arterial end *remained normal*.

3. *Increased osmotic pressure in the endolymphatic space caused by an excess of hydrophilic hyaluronic acid-protein aggregates will reduce the return-flow at the venous end regardless of the hydrostatic and osmotic pressures existing within the blood capillaries and in spite of retained physiological excretory function of the sac*

4 A blockage of the outlet from the endolymphatic space through the saccal duct (obliteration of the saccal duct) or excretory insufficiency of the saccal epithelium might result in an accumulation of the fluid and its dissolved solutes if such failure is not compensated by the blood capillaries of the microcirculation and the osmoregulators present in the endolymph.

Each of these mechanisms could represent a pathogenic component to hypertension in the endolymphatic space, and each of these factors might result from a different primary pathological process. Thus the etiology of endolymphatic hypertension cannot be explained by a single master mechanism. Because the control of the endolymph circulation results from a coexisting multiple mechanism, an intimate understanding of the formation and movement of the *endolymph* is the only way to clarify the etiology of an individual case of episodic labyrinthine vertigo related to endolymphatic hypertension.

Formation and movement of endolymph

It is commonly believed that the formation *of the fluid part* of the endolymph (water and electrolytes) *takes place by filtration from* the capillary beds of stria vascularis (Cawthorn, 1947 Shambaugh, Jr 1959 Lindsay 1946, Hallpike, 1938 Guild, 1927), from Shambaugh's glands (Shambaugh, Sr., 1908), *from capillaries of the spiral prominence*, and possibly from capillary arcades located in the basal membrane of scala media (Savin, 1965 Hawkins, 1964) these are the vascularized areas within the endolymphatic space (see Fig. 2). Moreover *radioactive isotope studies* of electrolytes in the endolymph and in the perilymph indicate (Rauch et al., 1963 1963 1958, Mnich, 1971) that sodium and potassium may pass through the Reissner's membrane from scala vestibuli into endolymph of the scala media against the high gradient of osmotic pressure. These observations therefore indicate that the electrolyte shift across the Reissner's membrane must be an *active* metabolic process selectively determined by the membrane. *One must not forget however a basic biologic fact that the shift of water and electrolytes from one compartment to another is controlled by two agencies. (1) osmotic equilibration between two neighboring compartments; and (2) an active metabolic property of proteins within the cell's*

disorder which when fully developed may completely incapacitate the patient fortunately such extremes of the disease occur infrequently and are interrupted by long remissions. Many patients in the milder stages of the disorder learn to live a more or less normal life by avoiding activities of precision while the symptoms last.

The reader may find a thorough discussion on the clinical picture of Menière's disease in articles written by Shambaugh Jr (1966 1968) and H. Williams (1965) The four cardinal manifestations of endolymphatic hydrops need not each one to be present in all cases. (1) The impairment of hearing which tends to fluctuate in the early stages, is probably an expression of changing osmotic or hydrodynamic pressure of endolymph within the cochlear canals the hearing is chiefly affected in the low tone range extending to the high tone range in the later stages of the disease. (2) Tinnitus may vary from mild buzzing to a roaring overpowering noise (3) Vertigo ranges from a mild unsteadiness lasting a few seconds to abrupt loss of balance, sometimes with falls followed by true vertigo which may completely incapacitate the patient for several hours or a day (4) Fullness in the homolateral ear or in the head is regarded by Lindsay (1946) as an important diagnostic symptom in endolymphatic hydrops. Other symptoms such as impairment of discrimination of speech recruitment of loudness, diplacusis, nystagmus nausea and vomiting are often associated with the active stage of the disease. All these signs, alone or in various combinations, are expressed in a fluctuating but steadily progressive course Long periods of mild manifestations may be interrupted by a vertiginous attack with or without other symptoms. In a complete remission which may occur "spontaneously or be induced by therapy all manifestations may subside completely or be grossly reduced in intensity Since spontaneous remissions are characteristic of this disorder there is always an element of doubt as to whether a remission was induced by therapy or not The reason for remission or relapse can be surmised but it can not be proven by our present methods because the etiology of endolymphatic hypertension is not completely understood

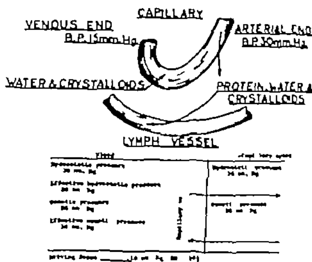


Fig 1 Diagrammatic presentation of dynamics of capillary circulation of tissue fluids in physiological conditions. (Reproduction by kind permission of the authors (Best & Taylor 1961) and publisher (Williams & Wilkins, 1961)).

Physiopathology of endolymph and endolymphatic system

The detailed mechanism of the physiopathology operating in the endolymphatic hypertension is far from being understood. The available data from animal experiments and from human pathology as well as from clinical symptoms [critically reviewed by Shambaugh, Jr (1966) and by H Williams (1965)] tend to indicate that no one single mechanism can be pointed out as the master mechanism" by which all cases of Menière's disease can be explained. Nevertheless, two topics are of fundamental importance

1 the basic principles controlling microcirculation of physiological fluids in all living tissues

2. the specific local anatomical and physiological factors controlling the microcirculation of the endolymph.

the endolymph is the epithelium which lines the endolymphatic sac. But McNally (1926) and later Lindsay et al. (1952) found in their experiments that the surgical removal of the sac and its duct had no significant influence on the pressure in the remaining intact endolymphatic space. However more recent studies by Kimura & Schuknecht (1965) by modified techniques demonstrated a delayed endolymphatic hypertension in some of the operated animals.

The hypothesis has been presented that the water of the endolymph with its dissolved or suspended molecules is excreted by the epithelium of the sac into the interstitial compartment of the loose connective tissue in the perisacal space, which normally contains a well-developed capillary network. A striking absence of the capillaries around the distended sac found in certain cases of Menière's disease was thought by Shambaugh, Jr (1968) to suggest a primary pathogenic cause of retention of endolymph which led to endolymphatic hydrops. Nevertheless, the same effect is more likely to be caused by a reverse mechanism: the primarily distended sac caused by the accumulation of the endolymph could occlude the capillaries by pressing and stretching them thus leading to an ischemia which, if persistent, might eventually lead to an ischemic atrophy of the perisacal connective tissue. Naturally such elimination of the capillary circulation from the perisacal connective tissue would aggravate a primarily existing endolymphatic hydrops.

The endolymph excreted and mixed with the perilymph in the perisacal space is next drained into the lateral cranial sinus (Guild, 1927), or mixed directly with the cerebrospinal fluid. The excretory action of the sacal epithelium must be regarded therefore as a specific function of its columnar epithelial cells (Guild, 1927). This biological function must be warranted by the general principles of physiologic energy exchange in which thyroid hormone, cyclic AMP, prostaglandins, etc. play an important role. The resorption of endolymph in the stria vascularis should be regarded, on the other hand, as the effect run exclusively by physicochemical forces.

The formation of endolymph in the endolymphatic space [corti-endolymph (Engstrom 1960), cochlear endolymph, sacal endolymph] should be viewed in identical terms as that of the formation of interstitial fluids in peripheral subcutaneous tissue, or of fluids in other serous cavities, such as joint cavities (Asboe Hansen, 1950). The composition of these fluids is somewhat similar to, but by no means identical with, the endolymph: the chief difference lies in the quality and the quantity of the proteins present in these fluids. Nevertheless, the hydrostatic and osmotic dynamics which control the circulation of such fluids are identical to those which control that of endolymph.

The protein moiety in the endolymph is formed by the epithelium lining of the endolymphatic space in a fashion similar to that of the lymph in the lymphatic spaces in the peripheral tissues, or to that of the serous fluids in other serous cavities, which is produced by fibroblasts in the former and by the epithelium in the latter instances. Diagrammatic presentation of the hydrostatic and osmotic forces which control the circulation of these peripheral fluids is shown in Fig. 1 (Best & Taylor 1961). The blood capillary loop shown in this diagram might represent a peripheral capillary bed of stria vascularis. The interstitial fluid formed in the peripheral tissues, for instance in the subcutaneous tissues, might correspond to the endolymph present in the scala media. The flow of the lymph in the peripheral lymphatic capillaries may be compared to the successive shift of the endolymph going in two directions, to the endolymphatic sac and to the stria vascularis. If the interstitial spaces of the peripheral tissues were locked up in such an area as the endolymphatic space, and the entire diagram considered to be a capillary bed located within the temporal bone—the formation, circulation and equilibration between the compart

structure which separate the two compartments.

Proteins and their derivatives are the living elements in the body which can either specifically interact or repel electrolytes, thereby either retaining them within the cytoplasmic medium (or fluid of one compartment) or rendering them available to osmotic shift. The shift of electrolytes from perilymph across the Reissner's membrane is the basis for the theory of "radial flow" of endolymph (Naftalin & Harrison 1958) in contradistinction to "longitudinal flow"—from stria vascularis to the sac. It has also been shown in animals (Rauch 1963 Mnich, 1971) that the radioactive cations which were shifted into endolymph from perilymph were rapidly eliminated into the general circulation via the venous end of the microcirculation of stria vascularis. In other words, the permanent residence of electrolytes within the endolymph is a secondary phenomenon which depends on the type of proteins present in the endolymph. The movement of the electrolytes from one compartment to another is physically carried out by the equilibration of the osmotic gradients in the adjacent compartments, and they are shifted *by a passive driving force*. But the availability of free electrolytes depends on their release from the conjugation with the protein molecules. Therefore there are two types of the endolymphatic hypertension (1) that which is *produced by the hyperosmolality of the endolymph resulting from the altered proteins* and this type anatomically does *not* produce a hydrops of the endolymphatic sac (2) that which is *produced by the hyperosmolality caused by abnormal proteins and bound to them electrolytes and this type of endolymphatic hypertension presents an endolymphatic hypertension with the distended endolymphatic space (hydrops).*

Perhaps at this point of the discussion it may be appropriate to emphasize the unique physio-chemical conditions existing within various compartments of the endolymphatic space. Although actual anatomical barrier is non-existing yet there are at least three (or more) different compartments, in two of which (sacal and cochlear) the composition of the endolymph differs significantly (Silverstein 1966 1971). The composition of the endolymph of the third compartment (cortilymph) is not as yet known in detail. The fact that the endolymph moves freely through both compartments, each retaining its specific characteristics, indicates that the element which controls the qualitative differences of endolymph in each compartment must exist within the endolymph itself such a specific action could only come from a protein moiety which differs in each compartment

The binding and freeing of water and electrolytes which permits the endolymph to be shifted (not flow!) must serve a useful purpose in the mechanism of hearing. The supply of essential nutrients by the endolymph for the organs of hearing and equilibrium is not only most logical but is also well-documented. However the discrepancy in the compositions of various endolymphs must also possess other than nutritional effects. Since the lymph from the various cochlear compartments differs significantly pathologic changes in the form of quantitative or qualitative alternations in proteins might therefore affect one cochlear compartment leaving the other two unaffected, or two of the three compartments could be simultaneously involved Therefore an increased hydrodynamic pressure located for instance in the cochlear duct might not necessarily cause a distension of the endolymphatic sac And a localized hyperosmolality of the cortilymph which might conceivably impede the mechanism of hearing, might not necessarily be reflected in a *hyperosmolality of the* cochlear or sacal endolymph If this is true a therapeutic drainage of the sac by fistulation or by enlargement of the space of the sacal environment might not affect the symptoms of every case of endolymphatic hypertension.

It has been believed (Guild, 1927 Lawrence et al. 1961) that the main resorptive area for

toneum (Kocen, 1952, Levine, 1960; Lange, 1944). Under same pathological conditions hyaluronate mucopolysaccharides may also accumulate in the vitreous of the eye (Larson, 1958; Dische & Zelmanes, 1955). The symptoms produced by expansion of fluids in the pleura, pericardium or peritoneum are less dramatic than in the endolymphatic space, because the expansibility of the endolymphatic space is, for practical reasons, nonexistent.

Circulation to and from the endolymph is another factor influencing the pressure in this organ. The water and water solutes flow in two directions, from the arterial end of the capillary beds of the stria vascularis to the venous end of the capillary beds of stria vascularis and to the endolymphatic sac; each route may take over the function of the other when an obstacle blocks its passage. The driving force of the in-and-out flow of water in the physiological state equals 10 mmHg (see Fig. 1). When the osmotic pressure of the endolymph rises, as the result of water binding by the hyaluronate mucopolysaccharides, the filtration of the water at the arterial end in the stria vascularis proceeds until the *osmotic and hydrostatic driving force* (20 mmHg) becomes equal on both sides of the capillary wall. On the other hand, increased osmotic pressure in the endolymph prevents the outflow from the endolymph normally generated by *osmotic driving force of the blood plasma proteins* at the venous end of the capillary loops in stria vascularis. In these circumstances maintenance of a near normal endolymphatic flow may rest on the excretory function of the sac epithelium, provided the action of hyaluronidase on the hyaluronate mucopolysaccharides will liberate free water. Thus, three factors ultimately control the physiologic flow of the endolymph.

(1) filtration rate of water and crystalloids at the arterial end (controlled by hydrostatic pressure) and their reabsorption rate at the venous end (controlled by the osmotic pressure exerted by plasma proteins)

(2) osmotic pressure of the endolymph itself

Table I. *Abnormal findings in metabolic survey of 250 patients with Menière's disease*

Normal values in brackets

	No. of cases with found abnormality	
P.B.I. – reduced (4–8 mcg %)	81	32.4
B.M.R. — reduced (±8)	190	76.0
Cholesterol — elevated (mg)	13.	52.8
Glucose Tolerance Test (low or nonabsorptive)	128	51.2
Creatinine — reduced (1 3 mcg)	16	6.4
Calcium — elevated (9–12 mg)	5	.0
Uric acid — elevated 2 5 mg)	115	46.0
Magnesium — reduced (as Mg ++ 1 –2.5 mg)	10	4.0
Protein (abnormal electrophoresis pattern)	175	72.0
Infections	228	91.0

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(3) the excretory clearance by the epithelium of the sac (the control of this function is not conclusively known).

The other factor which has been suggested as influencing the endolymphatic pressure and endolymph flow such as fluctuations in the hydrostatic pressure of the cerebrospinal fluid caused by changes of the position of the whole body from upright to recumbent and vice versa, should be regarded as transitory. Such an effect could exert a cushioning or "milking" effect of cerebrospinal fluid upon the sac to prevent transmission of changes in intracranial pressure to the inner ear which might disturb equilibrium (Allen, 1964). These effects cannot, however, have a continuous effect on endolymphatic pressure.

Rhythmic expansion and collapse of the endolymphatic pressure might also be caused

Fig 2 Diagrammatic presentation of factors controlling the circulation of endolymph. Hyaluronidase is secreted into endolymph by mast cells located in the membranes of the endolymphatic space (Poulsen, 1966) Reissner's membrane is permeable to water crystalloids and other small molecular substances (Naftalin et al. 1958 Mnich 1971) Anti-hyaluronidases penetrate into endolymph via capillarized organs in endolymphatic spaces. Free" water is presented as round circles, "bound water to hyaluronate mucopolysaccharides are presented as circles surrounded by interrupted lines.

Histamine and its analogue (Schayer 1964) acts on the pre-capillary sphincter thereby regulating hydrostatic pressure in the capillary bed and the rate of filtration through the capillary wall.

ments of the endolymph would still be controlled by the identical osmo-dynamic principles as when it represented the interstitial fluids or serous fluids in various serous cavities. However the composition of proteins in the endolymph (Silverstein 1971) indicates that it is not a simple transudate from the plasma and the difference from other serous fluids being probably because of the adaptation to the highly sensitive mechanics of hearing and equilibrium. *The protein moiety of the endolymph therefore acquires a specific significance in characterization of the endolymph and the whole problem of endolymphatic hypertension pivots on the nature of the protein.*

Role of protein in circulation of endolymph

Proteins may be secreted physiologically by the endolymphatic epithelium or may be exuded through the capillaries in an allergic, toxic reaction bacterial viral and fungal infection. There are three varieties of such proteins

(1) mucopolysaccharides of the hyaluronic acid order (large hydrophilic molecules)

(2) hyaluronidase an enzyme which depolymerizes hyaluronate mucopolysaccharides by splitting off the molecule of hyaluronic acid, thereby depriving them of hydrophilic properties

(3) anti hyaluronidases which control the action of hyaluronidase (not all anti hyaluronidases belong to proteins)

The accumulation of hyaluronate mucopolysaccharides in any serous cavity or tissue causes an accumulation of fluids. In myxedema such an accumulation might be found in the pericardium (Askensay 1898 Hurxthal 1935) in pleura (Farayoni, 1952) or in peri-

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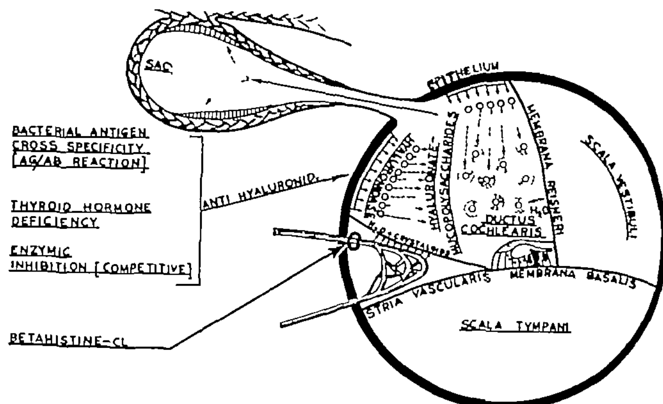


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Fig. 3. Histological section of normal human labyrinth stained with toluidine blue. Note deep blue-purple color of all components of the inner ear caused by staining with toluidine blue the free hyaluronic acid split from hyaluronat mucopolysaccharides. Free hyaluronic acid in the tissue proves the presence of the hyaluronidase action. 500.



Fig. 4. Histology of inner ear from a patient with labyrinth hydrops, stained with toluidine blue simultaneously with identical tissue from a normal ear (Fig. 3), 500. Note brown-yellow color of all structures of inner ear caused by the absence of *free* hyaluronic acid, which remained conjugated with mucopolysaccharides as the result of absence of action of hyaluronidase.

Table II *Endocrinopathies associated with 250 patients with Menière's disease*

	No. of cases	%	
Hypermetabolism	15	6.01	
Hypothyroid	12	4.8	88.8
Hypometabolic*	150	60.0	
Parathyroid	10	4.0	
Gonads	18	7.4	
Pancreas	15	6.4	
Adrenal[b]	8	3.2	
Pituitary	18	7.4	
Hypothalamic syndrome	168	67.2	
Unclassified	6	2.5	

PBI T4 normal BMR low cholesterol high low absorptive GTT
[b] Adrenogenital syndrome.

by fluctuations in the systolic and diastolic expansions of the arterioles and met-arterioles of the capillary beds in the vascularized organs of the endolymphatic space This effect could only represent an insignificant role which under physiological conditions could not produce a steady effect upon either the flow or the pressure of endolymph. This mechanism however might produce pressure "waves in the endolymph in pathological conditions such as arterial hypertension or in diseases connected with an increased pulse pressure such as aortic regurgitation or *thyrotoxicosis*. Transitory dizziness is oftentimes associated with these conditions, but such vertiginous symptoms cannot be classified as Menière's disease although transitory hypertension in the endolymphatic space is the cause of them. On the other hand, it is well known that Menière's disease is seldom associated with high blood pressure aortic regurgitation or thyrotoxicosis.

In an analysis of the *metabolic findings* recorded in our series of 250 patients diagnosed as Menière's disease (Tables I and II) a dysproteinemia of plasma proteins has been found a common feature The consistency in alterations in the quality of plasma proteins suggested that the primary pathogenic mechanism operating in patients with Menière's disease exists in the whole organism and inner ear pathology is only an extension of the pathology in which the whole organism is involved To substantiate such a contention we have studied the problem by two methods (1) *histochemical* of the cochlea from a normal and from an ear of a patient with Menière's disease and (2) *biochemical analysis of the inner ear fluids* and the blood plasma from the patient with Menière's disease as well as the plasma from a normal individual.

Histological section of a normal human ear (Fig. 3) and of an ear from advanced Menière's disease were simultaneously stained with toluidine blue which specifically stains free hyaluronic acid ("H") giving dark purple-blue color however if "H is conjugated with mucopolysaccharides this tinctorial property is completely lost. The large molecules of hyaluronate mucopolysaccharides possess a strong water-binding property (*hydrophylic*) whereas mucopolysaccharides without "H" depolymerized by enzyme *hyaluronidase* ("HD") lose this tinctorial property and become hydrophobic In Fig. 4 staining with toluidine blue gave yellow-brown color proving thereby complete absence of H as a result of the deficit of depolymerizing action of hyaluronidase Naturally the endolymph could not be demonstrated in this section because it was destroyed in process of preparation

The *biochemical studies* by electrophoretic technic (Fig. 5) the endolymph perilymph cerebrospinal fluid and plasma proteins from a patient with advanced Menière's disease and plasma proteins from a normal individual were analyzed. The details of the technic are described in the legend of Fig 5 In series C (endolymph) strip No 1 shows normal amounts of hyaluronic acid as compared with control series A strip No 1 but if acted upon with double amount of hyaluronidase in strip 4 in series C released hyaluronic acid from hyaluronate mucopolysaccharides grossly exceeded the quantities of H released in strip No. 4 of control series A. The H" of serum



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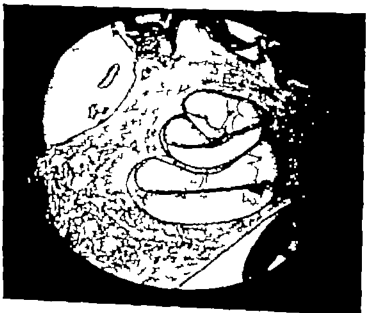


Fig. 4. Histology of inner ear from patient with labyrinth hydrops, stained with toluidine blue simultaneously with identical tissue from normal ear (Fig. 3), 500. Note brown-yellow color of all structures of inner ear caused by the absence of free hyaluronic acid, which remained conjugated with mucopolysaccharides as the result of absence of action of hyaluronidase.

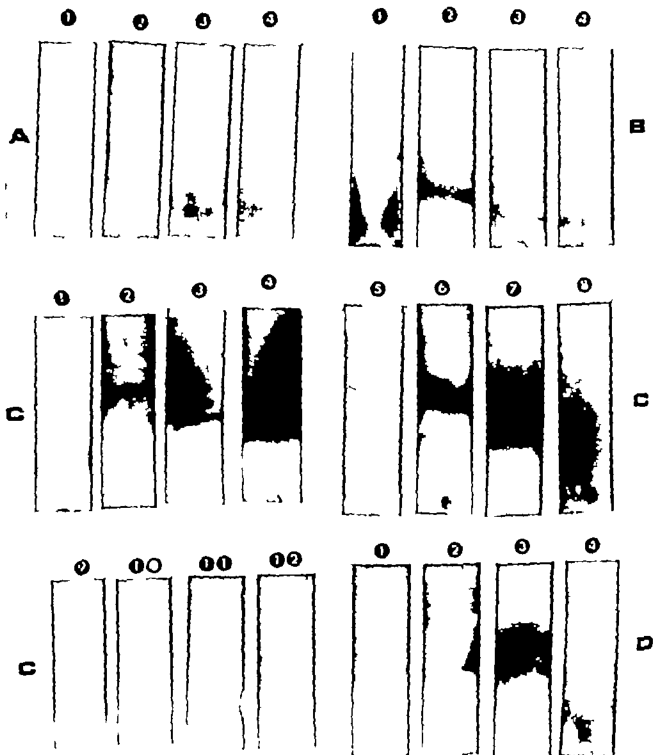


Fig. 5 Absence of free hyaluronic acid the fluid of inner ear (bound to h furon mucopolysaccharides) determined by electrophoresis showing similar conditions existing in the endolymph of patient with Menière's disease as those found in the circulating blood.

In series A 1 0.004 ml of 1 solution of h 1 nic acid (H) obtained from the h ma and (Sigma Chemical Co.) was selected arbitrarily as standard for colorimetric comparison with H of normal blood as well as with endolymph perilymph and blood from patient with d nced Menière disease and with H in CSF from a patient h some times before was decompressed by sh t between sac nd subarachnoid space. Electrophoresis made in llulose acet strips for H n pyridin formic id buffer pH 2.8 with 3.5 m pe t p and 150 for turning a used A nd O ge O with staining th aq sol 1 t Al ne blue

In series 4 1 0.004 ml of H w run alone n A 3 nd 4 the mount of hyal (HD) added w in g owing quantities H)

of the same patient with Menière's disease in series C in the strips 7 and 8 showed also a great accumulation of H⁺ equal to that found in the endolymph of the same patient. The cerebrospinal fluid from another patient with Menière's disease who was previously treated with shunt between the sac and subarachnoid space (series D) had similar accumulation of hyaluronate mucopolysaccharides which yielded similar amounts of H⁺ to those found in endolymph from patient with Menière's disease (series C).

We have concluded therefore that:

1. *Endolymphatic hypertension is an extension of a pathology involving the whole organism but clinical manifestations are dramatized in the inner ear*
2. *The primary cause of endolymphatic hypertension is a deficit of the hyaluronidase action caused by various etiological factors. This leads to accumulation of hyaluronate mucopolysaccharides in the endolymphatic space causing either a hydrops with all consequences of mechanical and nutritional sequelae or damaging the organs located in the endolymphatic space by dehydration exerted by hyaluronate mucopolysaccharides without forming a hydrops.*

The cause of the deficit of hyaluronidase action may originate in various mechanisms: (1) it could be either *hereditarily transmitted* and be triggered by any metabolic stress, or (2) an infection with a specific virus with the affinity to endolymphatic organs, or any infection, e.g. bacterial or fungal, or (3) toxic stresses such as hypersensitivity or chemical toxicity could eliminate hyaluronidase action (see Fig. 2). The other two factors, i.e., changes in the hydrostatic and/or osmotic pressure in the blood in the vascularized areas, or isolated dysfunction of the endolymphatic sac, could have only a contributory effect in most instances. Although neither increased hydrostatic pressure within the arteriolar capillaries nor hypoproteinemia in the blood can be regarded as a primary mechanism in endolymphatic hydrops, the artificial reduction of the head pressure in the capillaries and/or elevation of the osmotic pressure in the circulating plasma can be utilized *in pharmacological decompression* of the endolymphatic hypertension for symptomatic alleviation of symptoms of Menière's disease (see below).

Lindsay et al. (1947 1952) have ruled out the excretory failure of the saccal epithelium as a primary etiological mechanism of the labyrinthine hydrops by removal of sac with its duct in animals without causing significant changes in the endolymphatic pressure. Although in more sophisticated technic Schuknecht & Seifi (1963) and Kimura & Schuknecht (1965) by the removal of the sac have caused an experimental endolymphatic hypertension but only in a few animals of the whole series. Since the majority of the operated animals did not develop any signs of endolymphatic hypertension, those few positive ani-

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as 1 1 1 2, 1 3. The intensity of blue staining as approximately equal in all strips as in series A 1.
I series B (serum from normal person) the blue color slightly intensified with increased amount of "HD" added to serum shown in strips B 2, 3 and 4 as result of depolymerizing of free "H" from normal amounts of hyaluronate mucopolysaccharides present in normal blood by greater quantity of "HD".
I series C 1 (endolymph from Menière disease patient) the amount of depolymerised free "H" greatly increased with increased amount of "HD" added to endolymph proving that accumulated hyaluronate mucopolysaccharides required more "HD" action for the liberation of free "H".
I series C 5 6,7 and 8 (serum from the same patient) accumulation of hyaluronate mucopolysaccharides was equally high as those found in the endolymph (C 1,2,3 and 4).
I series D (CSF from patient with Menière's disease decompressed by shunt between sac and subarachnoid space) the conditions are similar to those in endolymph and serum from patient with Menière disease.
I series C 9 10 11 and 12 (perilymph from patient with Menière's disease) the amount of free "H" was found in much lesser quantities than those found in endolymph.

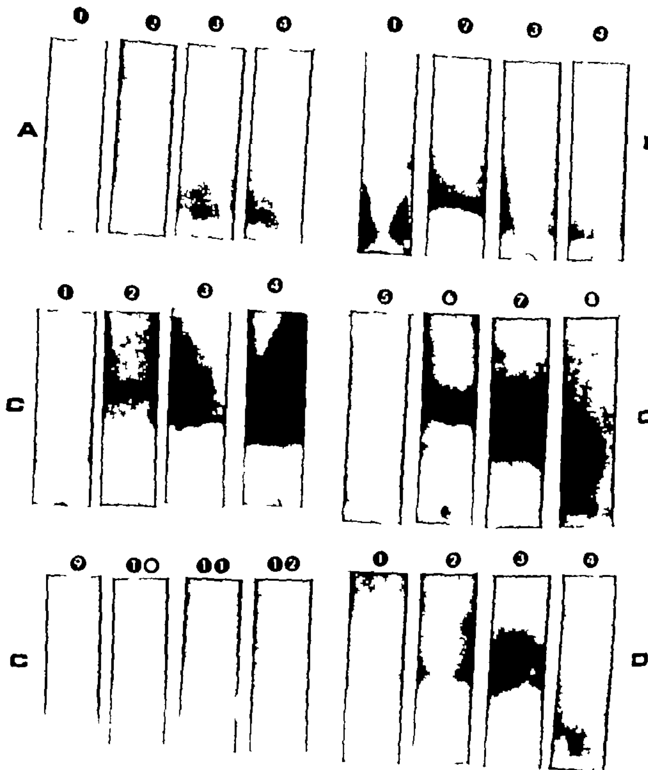


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thereby creating a deficit in depolymerizing action of normal mucopolysaccharides (*deficit of hyaluronidase*).

3. Production of structurally normal mucopolysaccharides in normal quantities which cannot be depolymerized by normal quantities of hyaluronidase on account of the high titer of anti-hyaluronidases, (*excess of anti-hyaluronidases*).

All three variants might exist either alone or in various combinations with the same consequences, that is, excessive binding of water thereby restricting the movement of the endolymph. Presence of the hydrated mucopolysaccharides which have not released the water is important because (1) the flow of nutrients to the organs located in the endolymphatic space is restricted, and (2) the hyperosmolality of the endolymph increases the pressure upon the organs located within the endolymphatic space. According to the prevalence of the individual pathogenic factors, the manifestations of the endolymphatic hypertension will vary from one case to another

Hyaluronidase/Anti-hyaluronidase ratio

The physiological depolymerizations of the hydrophilic mucopolysaccharides by hyaluronidase is controlled by numerous factors, biochemical, immunological, and endocrinological These factors are collectively designated as *anti-hyaluronidase effect* or *atio* The collective term of *anti-hyaluronidases* represents, therefore three groups of factors which may inactivate hyaluronidase (Giblan, 1951)

1 *Plasma anti-hyaluronidase* is physiologically present in the blood in varying concentrations (referred to as the titre of anti-hyaluronidase) Chemically it is a glycoprotein (Meyer et al. 1936) which inactivates the depolymerizing action of hyaluronidase by splitting of hyaluronic acid from hyaluronate mucopolysaccharides. The origin of plasma anti-hyaluronidase is not known. The physiological function of plasma anti-hyaluronidase is to maintain normal hydration of tissues and physiological fluids by inactivating the depolymerizing action of hyaluronidase or in turn, by withholding its inhibitory action, thus sustaining the tissue turgor and physiological volume of various serous fluids. A high titre of plasma anti-hyaluronidase may cause elastic edema (metabolic edema) or hydrops in serous cavities. Conversely a low titre of plasma anti-hyaluronidase leads to dehydration (metabolic dehydration). This form of anti-hyaluronidase travels electrophoretically with globulins, and acts by an immunological mechanism.

2. *A hormonal anti-hyaluronidase effect* may derive from a deficit of thyroid hormone (th.h.) which is required for the formation of proteins in general, and hyaluronidase in particular Thyroidectomized guinea pigs with fully-developed myxedema do not possess hyaluronidase at all these guinea pigs accumulate mucopolysaccharides in all organs. When a th.h. is adequately supplied hyaluronidase reappears and is followed by a normal rate of depolymerization of hydrophylic mucopolysaccharides (Asboe Hansen, 1954). Studies of the myxedematous guinea pigs by Poulsen (1966) and thyroidectomized sharks by Vilstrup (1954) have shown a high content of hyaluronate mucopolysaccharides in the endolymph, which are known to possess the water-binding capacity

3 *An immuno-chemical anti-hyaluronidase action* can result from compounds originating outside or inside the organism, such as bacterial toxins ("Spreading factor" (Duran-Reynolds, 1929)) protein catabolites in anaphylactic reactions, heavy metals, and the like.

Thus we regard a deficit in the depolymerizing action of hyaluronidase as the most common primary etiological mechanism of endolymphatic hypertension. Secondary factors such as increased capillary permeability in the stria vascularis or dysfunction of the excretory action of the sac and its duct (which might end in the obliteration of the saccal duct and vestibular aqueduct) increase the severity of the hydrops and may create an intractable form of the disorder

mals must be accepted as an experimental coincidence.

Normally in cats, the endolymph of the endolymphatic sac is more concentrated and possesses a viscous character as compared with endolymph from the cochlear duct, chiefly because of concentration of proteins is about forty times higher: potassium in turn is greatly reduced and sodium is increased (Silverstein, 1966). This suggests that the water and potassium are eliminated by the epithelium of the sac but this does not imply that an alternate route for the excretion of water and potassium from the endolymph by venous return in the stria vascularis does not exist. If sacal excretion is blocked as a result for instance of the obliteration of the capillary net caused by a distended sac, and simultaneously elimination of water by venous return is reduced on account of hypoproteinemia of the blood plasma, lost or reduced function of the sac might definitely aggravate an existing hypertension in the endolymphatic space. In other words, the insufficiency of the sac's excretory function may aggravate but not primarily cause endolymphatic hypertension or if it does, it occurs on rare occasions in conjunction with dysproteinemia existing in the whole organism.

The obliteration of the vestibular aqueduct found by Clemis & Salvassori (1968) is regarded by the authors as a contributory cause of the endolymphatic hydrops. Using radiological studies they found absence of the vestibular aqueduct in prevailing majority of true Menière's disease. On histological examination of temporal bone they found that the vestibular aqueduct was either completely obliterated or its location marked only by a filiform band of connective tissue. They have suggested that the absence of the vestibular aqueduct (and the sacal duct located in the aqueduct) might be explained by either an error in the embryological development or by disease after birth. Other workers found no pre-existing developmental anomaly (Anson 1968) and no abnormality of petrous bone from patients with Menière's disease was found (Schucknecht 1968). It is therefore more probable that the absence of vestibular aqueduct (and sacal duct) is a secondary phenomenon caused by primary pathogenic noxa which on the other hand, caused endolymphatic hypertension and, later inflicted its destruction followed by a complete calcification of the destroyed sacal duct or the debris of the wall of the sacal duct became organized into the band of filiform connective tissue.

Physiopathology of mucopolysaccharides in endolymph

Metabolic studies on patients with Menière's disease shows that reduced oxygen uptake and dysproteinemia in the blood plasma (Table I) are the commonest metabolic error. These two basic physiological factors control normal metabolic processes of all organs. The same pathogenic mechanism which caused abnormality in these two physiological factors also act on the formation and function of the endolymphatic space. The endolymph obtained from the few patients with Menière's disease available for such analysis showed the same dysproteinemia in the endolymph as in the plasma proteins. This was revealed in the electrophoretic pattern as an excess of hyaluronate mucopolysaccharides (Fig. 5). Therefore a discussion of the condition under which mucopolysaccharides might be expected to accumulate in the endolymph is needed to clarify the pathogenic mechanism operating in endolymphatic hypertension.

Three factors can lead to the accumulation of hydrophylic mucopolysaccharides in body cavities (including endolymphatic space) and change their function *without necessarily increasing their volume*.

1. Excessive production of hydrated form of mucopolysaccharides resistant to the depolymerizing action of normal hyaluronidase (*excess of stable hydrated mucopolysaccharides*).

2. Reduced production of hyaluronidase

thereby creating a deficit in depolymerizing action of normal mucopolysaccharides (*deficit of hyaluronidase*).

3 Production of structurally normal mucopolysaccharides in normal quantities which cannot be depolymerized by normal quantities of hyaluronidase on account of the high titer of anti-hyaluronidases, (*excess of anti-hyaluronidases*).

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An endocrinological survey of 250 patients with Menière s disease (Table II) has revealed deficiency in th.h. in one form or an other in 89 2% of all cases studied. It is therefore suggested that the th h. deficiency led to a general deficiency of protein formation and to an inadequate function of hyaluronidase. A state of molecular disease of proteins coexists in other organs but the degree of dysproteinemia is either not sufficiently advanced or the methods used are not sensitive enough to demonstrate anatomic changes and/or dysfunction of other organs.

The hypometabolic states which contribute to a low titre of hyaluronidase in the endolymph are associated with other factors which might secondarily influence the depolymerizing action of hyaluronidase. For example, a *deficit of th.h. facilitates invasion of the or* ganism by various living pathogens. Hyaluronidases of bacterial or fungal origin (also called "spreading factor" (Duran-Reynolds, 1929)) may act as an antigen and induce the formation of specific antibodies directed against the bacterial hyaluronidases. A subsequent Ag/Ab reaction would immobilize the bacterial hyaluronidase. By cross-specificity these same bacterial anti hyaluronidases may act upon the organism s own hyaluronidase and block *its depolymerizing action*. Pathogens which exist in the body in an apparent state of physiological coexistence with the host also may produce toxins which act as enzymic inhibitors. These enzymic inhibitors may invade the organism in *sub-toxic doses* without causing a local reaction at the portal of their entry Constant flooding of the organism by enzymic inhibitors from subclinically involved organs is often not appreciated either by the patient or by his physician be cause the cause and *effect relationship* is not apparent for instance between Menière s disease and chronic prostatitis or chronic cervicitis.

In our series of 250 patients with Menière s disease the incidence of attenuated chronic infections was 91 2 % (Table III). About half of these were prostatic or vaginal infections. Control of the infections by specific anti-bacterial or anti fungal agents along with hormonal replacement resulted in remission of the Menière s disease which promptly relapsed when local or systemic acute infectious agents were discontinued Hormonal replacement in these patients was insufficient for control of the disease

Table III ?50 *cases of Menière s disease surveyed and managed by immuno-metabolic technique*

123 males / 1 7 females

		No of cases	
Infection			
Upper respiratory tract			
Male	48	113	44.9
Female	65		
Sex organs			
Male	70	115	46.0
Female	45		
Metabolic error			
Thyroid hormone			
Male	108	[illegible]3	89.[illegible]
Female	115		
Other			
Male	31	85	34.0
Female	54		
None			
Male	[illegible]	8	3.2
Female	6		
Allergy			
Male	78	17[illegible]	68.8
Female	94		
Response to treatment			
Complete			
Male	38	70	[illegible]8.0
Female	3		
Partial remission			
Male	67	137	54.8
Female	70		
Negative			
Male	17	4	16.8
Female	[illegible]5		

Bacterial and fungal toxins are not the sole inhibitors which may affect the depolymerization of mucopolysaccharides by hyaluronidase. A group of organic and inorganic compounds either penetrating the organisms from without or originating inside the body may also interact with and inhibit the depolymerizing action of hyaluronidase These

reactions are usually of competitive character and as such, they are reversible. To these enzymic inhibitors belong compounds such as heparin when used in higher than physiological concentrations, hyaluronic acid in the acetylated, sulfated, and nitrated salts—sulfated esters of cellulose xylan, aliphatic alcohols, anionic dyes, bile salts, sulfated steroids, derivatives of diphenols, aurin, tricarboxylic acid, di- and trivalent metals, especially Fe Fe^{++} Cu Zn and half-products of protein metabolism, such as toxic catabolites originating in anaphylactic reactions (Meyer et al. 1936).

Anatomical changes in inner ear in Menière's disease

The histologic appearance of a normal labyrinth from an ear involved in "labyrinthine hydrops" is presented in Figs. 3 and 4. The staining of both sections with toluidine blue indicates a low content of hyaluronate mucopolysaccharides in the petrous bone, and in the membranes of the endolymphatic system in the normal ear (Fig. 3). In Fig. 4 the advanced stage of Menière's disease shows the destructive effects of endolymphatic hypertension with accumulation of hyaluronate mucopolysaccharides in the tissues of the inner ear the endolymph is not visualized in both sections. Both sections were stained by the same technic simultaneously The much stronger purple color of all tissues in Fig. 3 (free hyaluronic acid) results from the adequate depolymerization of mucopolysaccharides by hyaluronidase predominance of pale-yellow background in Fig. 4 is caused by the accumulation of hyaluronate mucopolysaccharides resulting from a low titre of hyaluronidase action causing a deficit in depolymerization.

The gross and microscopic pathological changes found in endolymphatic hydrops (Lindsay et al., 1952 Mygind, 1946 Silverstein, 1966) can be classified into two categories. (1) those caused by a deficit of nutritional factors, and (2) those resulting from mechanical effects, with the qualification that the first could result from the second. Degenerative changes found in the hair cells of the organ of Corti, in the epithelium of the macula and that of its cristae of the semicircular canals, in the stria vascularis and in the ganglionic cells in the spiral ganglion (not always demonstrable) can be explained either by a dysproteinemia resulting from a primary pathological process involving the entire organism or from nutritional defect resulting from local ischemia caused by increased pressure within the endolymphatic space.

Other pathologic findings such as distention and rupture of Reissner's membrane, distension of the whole endolymphatic system with obliteration of the perisacal space which normally contains a rich blood supply result from the increased pressure in the endolymphatic space, etc. Excessive endolymphatic pressure which causes local ischemia leads, in turn, to tissue hypoxia, degenerations, and eventually to atrophy this might also be the mechanism of the atrophy of sacal duct followed by obliteration of the vestibular aqueduct (Clemis & Salvassori, 1968).

The chances for the restitution of the atrophic hair cells in organ of Corti to a normal function in the inner ear are minimal or none when morphologic changes have advanced into the irreversible state this applies also to the rupture of Reissner's membrane (some authors maintain that such repeated rupture and healing of Reissner's membrane, marks the remissions and relapses of Menière's disease (Lawrence & McCabe, 1959)) and obliteration of the perisacal space (Shambaugh ascribes etiological value of the loss of capillary network in the perisacal connective tissue (1966)). If the organic changes of the endolymphatic system is still in a reversible stage, a properly selected therapeutic management will arrest the progress of the disease or induce a partial or complete remission, regardless of the duration of the disease. Unfortunately since information on morphologic changes is not available to the clinician, it is impossible to predict the end result of therapy

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In our series of 250 patients with Menière's disease, the incidence of attenuated chronic infections was 91.2% (Table III). About half of these were prostatic or vaginal infections. Control of the infections by specific anti-bacterial or anti-fungal agents along with hormonal replacement resulted in remission of the Menière's disease which promptly relapsed when local or systemic acute infectious agents were discontinued. Hormonal replacement in these patients was insufficient for control of the disease.

Bacterial and fungal toxins are not the sole inhibitors which may affect the depolymerization of mucopolysaccharides by hyaluronidase. A group of organic and inorganic compounds, either penetrating the organisms from without or originating inside the body may also interact with and inhibit the depolymerizing action of hyaluronidase. These

Table III. 250 cases of Menière's disease surveyed and managed by immuno-metabolic technique
123 males / 127 females

		No of cases	%
Infection			
Upper respiratory tract			
Male	48	113	44.9
Female	65		
Sex organs			
Male	70	115	46.0
Female	45		
Metabolic error			
Thyroid hormone			
Male	108	223	89.2
Female	115		
Other			
Male	31	85	34.0
Female	54		
None			
Male	2	8	3.2
Female	6		
Allergy			
Male	78	172	68.8
Female	94		
Response to treatment			
Complete			
Male	38	70	28.0
Female	32		
Partial remission			
Male	67	137	54.8
Female	70		
Negative			
Male	17	42	16.8
Female	25		

clinicians, (Cawthorn, 1947 Shambaugh, Jr 1959 Godlowski, 1952). In evaluating any type of episodic vertigo, a careful study of all potential sources of infection is essential. Throat, nasal and paranasal cavities, mouth, prostate, vagina, cervix and adnexa, may harbor *organisms* the products of which inactivate hyaluronidase and affect osmotic equilibrium in the entire organism, even though the presenting clinical symptoms may be limited to endolymphatic hypertension. The pathogens in the suspected areas, therefore, should be routinely investigated and their sensitivities toward antibiotics determined.

Any coexisting or suspected allergic hypersensitivities, particularly food allergy should be investigated because the products of allergic proteolysis (toxic catabolites) may also act as inhibitors of the depolymerizing action of hyaluronidase. Offending antigens may be detected by careful history intracutaneous diagnostic tests, cytotoxin food tests (Rinkel et al. 1951 Rinkel, 1962 and 1963 Rinkel et al. 1964) elimination diets with subsequent feedings" (Randolph, 1962) or by Intracutaneous Food Provocative Tests (Rinkel, 1962, Rinkel et al., 1964). For details see Chapter 28, *Allergy and Anaphylaxis as Metabolic Error* (Godlowski, 1962) and comprehensive summary in Tables IV and V

IMMUNO-METABOLIC THERAPY OF MENIERE'S DISEASE

The therapeutic aims in Menière s disease is the restoration of the osmotic pressure within the endolymphatic space to its physiological values. Only one factor can control the increased hydrodynamic and hyperosmotic pressures of the endolymph, that is restitution of the depolymerizing action of hyaluronidase. Retention of water and electrolytes are secondary factors which can be controlled by the specific action of the hydrophylic mucopolysaccharides. However three subsidiary factors may aggravate the primary etiologic mechanism which may on rare occasions, play a primary role in the rational treatment of Menière s disease

(1) excretory insufficiency of the endolymphatic sac with coexisting obliteration of sacal duct,

(2) fluctuations in the driving pressure within the arterial end of the capillary bed in stria vascularis, or reduction of the osmotic pressure of the blood plasma at the venous end;

(3) capillaritis involving the capillary net work in the stria vascularis.

Keeping in mind the main factors controlling the flow of endolymph and endolymphatic pressure, the therapeutic approach to endolymphatic hydrops runs along two lines:

(1) Elimination of the etiological factors which are responsible for *molecular disease of proteins* and the consequent hyperosmolality of the endolymph.

(2) Decompression of endolymphatic hypertension by medical and/or surgical procedures.

I. Etiological therapy of Menière's disease

Since Menière s disease is a local expression of a molecular disease of proteins involving the whole organism therapeutic efforts should be concentrated primarily upon the correction of the metabolic error Only then will reduction of endolymphatic hypertension obtained by surgical or pharmacological methods have a reasonable chance of staying in permanent remission. There are no specific therapeutic agents, save hyaluronidase, which selectively act upon the hyaluronate mucopolysaccharides. Only hyaluronidase depolymerizes the water-binding property of hyaluronate mucopolysaccharides within the endolymphatic system. So far therapeutic attempts to use hyaluronidase directly to reduce pressure within the endolymphatic space have failed completely (Marco *et al.*, *1956*). Deposited hyaluronidase in the middle ear of normal guinea pigs with the hope that it would diffuse into the endolymph was bound to *fail*

Clinical Management of Menière's Disease

INTRODUCTION

Clinical management of Menière's disease consists of (1) *diagnostic determination* of the primary cause which has initiated the hyper tension in the inner ear (2) administration of *specific therapy*

In most cases of Menière's disease two or three avenues must be employed simultane ously in the management of the patient in order to obtain maximal therapeutic success. The rational management of Menière's disease requires a close coordination of at least two specialists the otologist who possesses the informative diagnostic armamentarium and surgical skill necessary for a mechanical de compression or for destructive surgery but who is usually not sufficiently experienced to interpret the immunological and metabolic er rors or to administer the endocrine and meta bolic agents necessary to correct the primary molecular disease Endolymphatic hydrops is an immuno-metabolic disease just as is e.g diabetic retinopathy Both these conditions must be managed by a physician with a wide and profound immuno-metabolic background and experience who cooperates with the otologist or ophthalmologist respectively in order to maintain the patient in remission The qualified internist usually is not sufficiently skilled in diagnostic and therapeutic otologic procedures to differentiate the type of inner ear disease present or to evaluate objectively the progression or the remission of the disorder The general practitioner is neither equipped for otologic diagnosis nor for im muno-endocrinologic evaluation, although he is usually competent to carry out the therapy outlined by his colleagues.

DIAGNOSTIC EVALUATION OF MENIERE S DISEASE

The history if taken carefully may in many instances, reveal a connection in time between the onset of manifestations of endolymphatic hypertension and an infection. Mild infection especially could have been as consequential in precipitating endolymphatic hydrops as the severe forms. Not unusually "mild colds or "sore throats, cystitis or cervicitis indicate the avenue along which the diagnostic investigations should proceed Physical examination must include a complete otological study such as the hearing loss, functional otological tests consisting of determination of air and bone conductions, speech discrimination Bekesy audiometry caloric tests, and electronystagmographic. Study of labyrinthine function on the primary examination determines the form and the degree of the disease on which progress of the therapy is assessed

Further diagnostic studies are performed according to the immunometabolic survey as outlined in Volume 2 "Allergy and Anaphylaxis as Metabolic Error (Godlowski 1962) and summarized in Tables IV and V Proteins, carbohydrates, fat water and electrolytes must be investigated from as many angles as are available Particular attention must be paid to the thyroid profile keeping in mind the basic rule that a single test for thyroid function cannot satisfactorily evaluate the th h status even if a positive result is obtained.

The importance of bacterial toxins and antigens from acute or chronic inflammatory foci in precipitating endolymphatic hypertension has been strongly emphasized by many

exchange by the products of inflammation. Inflammations of bacterial, viral, and fungal origin, or those of allergic toxic, or physicochemical nature, possess one metabolic mechanism in common, i.e. liberation of products of incomplete catabolism of proteins which possess a variety of inhibiting effects on the enzymic systems. Each type of inflammation, however requires a specific therapeutic approach.

A. *Administration of broad-spectrum antibiotics or chemotherapeutic compounds* selected, if possible, by sensitivity tests in vitro The effective dose of the selected antibiotic should be given for at least seven to fourteen days, thereafter a similar dosage should be repeated during periods of seven days each month for six to twelve consecutive months. In resistant infections, combinations of antibiotics with sulfa compounds may circumvent mutation with the formation of new strains of pathogens resistant to the antibiotics being given.

B *Administration of local antiseptics to actively infected organs or those which are harboring them* Latent infections existing in organs which are regarded as "non-sterile" may create a hazard to patients with endolymphatic hydrops. Such infections are most apt to exist in mouth, throat, prostate vagina, or rectum. They require continuous control by an antiseptic which will restrict the growth of pathogens without obviously affecting the tissues of the host. I use Phenex Antiseptic® (Phenex Laboratory Chicago Illinois) as the antiseptic of choice for the following reasons

(1) Phenex Antiseptic does not damage the tissues of the host, but rather stimulates production of normal granulation tissue, and speeds up epithelization of ulcers and wounds

(2) it is anesthetic, analgesic, antipruritic, and deodorizing;

(3) it penetrates rapidly into the deeper layers of skin and mucous membranes,

(4) it is available in forms applicable to many infected organs, either as a solution, a lotion, an ointment, a nasal or throat spray or a rectal or vaginal suppository

(5) it possesses a wide spectrum of bactericidal and fungicidal effects.

In my patients with endolymphatic hypertension (see Table III) a chronic asymptomatic prostatitis was frequently found. Chronic prostatitis with or without hypertrophy is frequently found in men over forty Mild infections of the vagina, cervix and adnexa were also frequently found in women with Menière's disease. These infections must be energetically treated. Unfortunately the role of infection in the pathogenesis and therapy of endolymphatic hypertension is rarely sufficiently appreciated or given adequate therapeutic management by otologists.

C. *Anti-inflammatory steroids (glucocorticoids) as non-specific adjunctive therapy* The therapy with anti-inflammatory steroids should be initiated as soon as the diagnosis of endolymphatic hypertension is established and continued as long as the acute symptoms persist These steroids must be given along with other stress-relieving agents such as antimicrobial drugs when infections are present, and with other items of immunometabolic management. When the initial symptoms begin to subside the dosage of the steroid is gradually reduced while the other therapeutic agents are maintained. In moderately severe cases of endolymphatic hypertension, the average duration of steroid therapy is six to ten weeks. During this time the patient must be carefully supervised, at weekly intervals or oftener for side-effects such as cardiovascular embarrassment progressing osteoporosis, activation of peptic ulcer or other sources of gastrointestinal bleeding. In refractory cases, a maintenance dose of steroids may be required.

Prednisolone or its derivatives, is the preferable form of steroid because its sodium-retaining effect is less pronounced, and its anti-inflammatory effect is more marked than naturally occurring cortisol or hydrocortisol. The initial dose of prednisolone for adults

because the osmotic pressure within a normal endolymphatic system is higher *than that outside* the round window. Still less *chance of* success had the attempt to reduce the endolymphatic pressure in human endolymphatic hydrops by the deposition of hyaluronidase outside the round window (Wölger 1942) because *the osmotic pressure of the endolymph* in endolymphatic hydrops is much higher than it is under normal conditions so that the desired diffusion of hyaluronidase into endolymph is obviously impossible. In my own experience, intravenous infusion of five million units of hyaluronidase ("Wydase" Wyeth Company) during a six hour period resulted in the elimination of all four intractable symptoms (deafness vertigo tinnitus, and fullness *in ears) only for* the initial hour. Soon all manifestations returned to their previous severity and remained unchanged for the whole *period of* therapy during the next ten days *(Godlowski unpublished)*. Most likely the failure of intravenous therapy with hyaluronidase was caused by:

(1) a rapidly rising anti hyaluronidase titer in the blood inactivating the circulating exogenous hyaluronidase

(2) rapid distribution of the infused hyaluronidase through the extra-cellular compart*ments of the entire organism reducing the* circulating hyaluronidase to an insignificant quantity except for the first few minutes when the penetration of the endolymphatic space via the stria vascularis did occur (Meyer et al. 1936)

Because the diagnostic methods in use at present fail to elucidate the primary cause of the pathology in most cases of endolymphatic hypertension the clinical management must therefore be

(1) multilateral, encompassing all the possible pathogenic factors discussed above

(2) long term sometimes extending over the patient's whole life

The possibility that the symptoms of endolymphatic hydrops must be controlled for life has to be kept in mind by both the physician and the patient. Often both physician (particularly the otologist) and patient are *over-anxious* to obtain immediate relief which may oc*cur only in* exceptional instances. Sometimes instances of acute or subacute infections, allergic hypersensitivity reactions, or exposure to *enzymatic inhibitors*, when promptly recognized and *effectively treated will* restore the osmotic balance in the endolymphatic *space within* a few days. A complete perman*ent remission* can occur only if an anabolic supplement is adequately provided. In contrast to such cases, a spectacular improvement after surgery (chiefly in the vertiginous attacks, but less *often* in hearing and tinnitus) may restore the patient to a more or less *normal* life without eliminating the disease. These postoperative patients with a complete or partial remission still require continuous therapy otherwise they will experience a relapse of symptoms. It is often difficult, in these circumstances, to convince the patient, who is now without obvious complaints or the surgeon who is credited with the spectacular re*mission that the primary metabolic error* is still present and should be treated.

Immuno-metabolic therapy of endolymphatic hypertension consists of four items of equal importance. In intractable cases all four agen*cies should be employed concurrently*

(1) disposal of inflammatory stress

(2) supplementation with anabolic agents

(3) suppression of the property of hypothalamus to redistribute those pathologic stimuli which derive from the target organs

(4) allergic hyposensitization using optimal dosage technique

The details of this multilateral therapy are presented in Part VI of Volume 2 of *Allergy and Anaphylaxis as Metabolic Error* (Godlowski, 1965) however a brief summary of the management is presented below in Tables IV and V

1 *Disposal of the inflammatory stress*

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tion of T3 upon the endogenous output of TSH (Godlowski, 1965). The initial dosage of T4 is 0.05 mg or less once daily but gradual increase in dosage to the level of tolerance may be required. The duration of therapy with thyroid hormone depends on the *form* of thyroid hormone deficiency: *primary hypothyroidism* requires permanent administration of a maintenance dose for life (Godlowski 1965). In children and young adults, the dosage of th.h. is more liberal nevertheless, strict supervision is always indicated. T3 up to 25 mcg three times daily along with T4 up to 0.2 mg, or more, is the usual starting dose.

In *secondary hypothyroidism* TSH (5 to 10 units) or crude anterior pituitary extract (1 ml) is initially administered in weekly intramuscular injections together with other parenterally administered anabolic agents. When initial manifestations subside, the TSH injection should be extended to monthly intervals or as is required.

(7) *Male and female sex hormones.* Male hormone has a strong anabolic property in both sexes however in women it may have an untoward influence on the menstrual cycle or produce undesirable virilizing side-effects. Male hormone is given as *testosterone* preferably in an aqueous suspension to adult males in doses of from 25 to 50 mg injected intramuscularly once or twice weekly using the same syringe containing the other anabolic agents. In adult females, the dosage of testosterone should fluctuate between 5 and 25 mg in weekly injections mixed in the same syringe *with estrogens (10 000 to 20 000 units)* but avoiding the hormones in the week of expected menstruation. In men, estrogens are given in amounts which may fluctuate between 5 000 and 10 000 units a week. Feminizing effects or sexual impotence may occasionally become a side-effect of this therapy in which case the estrogens must be completely omitted. Adolescent and preadolescent children of both sexes are treated with sex hormones by an identical technique: testosterone 5 to 10 mg with estrogens with 5 *to* 10 000 units are injected intramuscularly weekly with other anabolic agents mixed in the same syringe. If masculinizing or feminizing effects complicate the therapy the dosage of the heterosexual hormone should be reduced or eliminated, and the isosexual hormone should be increased. Precosity occasionally may occur which, however spontaneously disappears on discontinuation of sex hormone therapy

The oral or injectable anabolic steroids (derivative of sex hormones) can replace sex hormones, as they fully retain the anabolic property but the sexual effects are grossly reduced these are: Nilevar®" Searle and Company "Winstrol®" Winthrop Laboratories, Durabolin®" Organon, Inc. etc. They can be used in both males and females, adults and children.

B. *Anabolic vitamin supplement* Vitamin B-Complex with ascorbic acid such as Vicam®" Marion Laboratories, Folbesyn®" Lederle Company: Berocca with C®" Roche Company: etc. are routinely given intramuscularly once a week mixed with other anabolic agents with addition of 1 ml 2% Pontocaine or any local anesthetic. Oral administration of vitamins in patients *of the older age* group is not recommended because of the frequency of malabsorption from the gastrointestinal tract secondary to senile atrophy and amyloidosis of the intestinal mucosa *and* pancreas (Schwartz, 1965). These changes are often found to be associated with dysproteinemia and tend to prevent utilization of ingested vitamins. Lipoflavonoids *supplemented with the vitamins* of the rest of B-complex and Vitamin C when applied as a sole therapy of endolymphatic hypertension has been shown (Williams, 1963) to be effective only in selected *forms* of Ménière's disease (Shambaugh, 1963 Derlacki, 1965). The rationale of large doses of anabolic vitamins in the therapy of endolymphatic hypertension is based on their mediating role in the anabolic action of hormones and enzymes which participate in the formation of proteins.

C. *Anabolic mineral supplement* Minerals

should be 5 mg three times daily only in rare instances should the daily dosage exceed 30 mg If side-effects appear during this short period of therapy the dosage must be drastically reduced by at least, two-thirds, and anabolic agents (see below) applied in double dosage If cardiovascular manifestations of a more serious nature appear or bleeding from the gastrointestinal tract occurs, steroids must be discontinued immediately and appropriate therapy instituted (Godlowski 1965)

Since the exact mechanism of cortisol action is not finally established the rationale for steroid therapy in endolymphatic hypertension is also not clear The available evidence indicates that normal proteolysis may be effectively enhanced by cortisol (Godlowski 1952) It is believed that normal proteolysis can be promoted by cortisol and that toxic catabolites, which inhibit hyaluronidase are catabolized to non toxic derivatives. If this is true, the depolymerizing action upon the hyaluronate mucopolysaccharides of the hyaluronidase within the endolymphatic space (and by that matter anywhere else in the body) may be restored and so the hyperosmolality of the endolymph thereby reduced

2. *Supplementation with anabolic agents*

Supplementation of the erratic metabolism with anabolic agents is a powerful instrument in the treatment of endolymphatic hypertension Anabolic agents consist of anabolic hormones, anabolic vitamins and minerals essential proteins although not anabolic agents per se represent the building material from which new proteins are formed therefore they are included in the anabolic supplementation therapy of endolymphatic hypertension.

A *Anabolic hormones* Supplementation of abnormal metabolism with anabolic hormones is accomplished with thyroid hormones and male and female sex hormones hormones which stimulate their production although non-anabolic per se (TSH FSH and chorionic gonadotropins) are briefly discussed here because they exhibit an anabolic effect as an end result.

(1) *Thyroid hormones.* Thyroid hormones are divided according to their physiological actions

(a) *a short-acting form* L-tri-iodothyronine (T3 Cytomel® *Smith Kline* and French Laboratory) supplied in tablets containing 5 mcg and 25 mcg respectively

(b) *a long-acting form* levorotary sodium tetraiodothyronine (thyroxine T4—Synthroid® *Flint Laboratory*) *in tablets* containing 0 025 0 05 0 1 0.15 0.2 0.3 0 4 and 0.5 mg—"Letter' ® Armour Company (in similar dosage), or its analogues

(c) *a long-acting dextrotary sodium tetraiodothyroxine* (D-T4—Choloxin® Flint Laboratory) in tablets containing 2 and 4 mg

Desiccated thyroid in any form is a less desirable way of medication because thyroglobulin (or any iodinated tyrosine compound) acts only if it is converted into T4 by specific hydrolysis which is often faulty furthermore, there are cases on record of antibody formation against thyroglobuline during treatment with desiccated thyroid tablets (Godlowski 1965) Two forms of thyroid hormone medication have recently appeared on the market "Thyrolar ® (Armour Company) and Euthroid ® (Chilcott Warner Laboratory) Both forms contain combination of T3 with T4 in various proportions. The advantage of such combination is that one tablet contains both forms of thyroid hormone short and long acting. The disadvantage, on the other hand of such a combination is that the combination of the two forms of thyroid hormone eliminates the therapeutic maneuverability if one of them is not well tolerated or is not required.

The dosage of T3 in adults over forty years is 5 μg three times daily if well tolerated, and the larger quantities are required for the therapeutic effects, then a gradual step-up in dosage to the level of tolerance may be indicated. In the same age group, T4 is given along with T3 to prevent the suppressive ac

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C. *Anabolic mineral supplement.* Minerals

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Since the exact mechanism of cortisol action is not finally established the rationale for steroid therapy in endolymphatic hypertension is also not clear The available evidence indicates that normal proteolysis may be effectively enhanced by cortisol (Godlowski 1952) It is believed that normal proteolysis can be promoted by cortisol and that toxic catabolites, which inhibit hyaluronidase, are catabolized to non-toxic derivatives. If this is true the depolymerizing action upon the hyaluronate mucopolysaccharides of the hyaluronidase within the endolymphatic space (and by that matter anywhere else in the body) may be restored and so the hyperosmolality of the endolymph thereby reduced

2. *Supplementation with anabolic agents*

Supplementation of the erratic metabolism with anabolic agents is a powerful instrument in the treatment of endolymphatic hypertension. Anabolic agents consist of anabolic hormones, anabolic vitamins and minerals, essential proteins, although not anabolic agents per se represent the building material from which new proteins are formed therefore they are included in the anabolic supplementation therapy of endolymphatic hypertension

A *Anabolic hormones* Supplementation of abnormal metabolism with anabolic hormones is accomplished with thyroid hormones, and male and female sex hormones hormones which stimulate their production although non anabolic per se (TSH FSH and chorionic gonadotropins) are briefly discussed here because they exhibit an anabolic effect as an end result.

(1) *Thyroid hormones* Thyroid hormones are divided according to their physiological actions.

(*a*) *a short-acting form* L tri iodothyronine (T3 Cytomel® Smith, Kline and French Laboratory) supplied in tablets containing 5 mcg and 25 mcg respectively

(*b*) *a long-acting form* levorotary sodium tetraiodothyronine (thyroxine T4—Synthroid® Flint Laboratory) in tablets containing 0.025 0 05 0.1 0.15 0.2, 0.3 0.4 and 0.5 mg— Letter ® Armour Company (in similar dosage) or its analogues

(*c*) *a long-acting dextrotary sodium tetraiodothyroxine* (D-T4—Choloxin® Flint Laboratory) in tablets containing 2 and 4 mg.

Desiccated thyroid in any form is a less desirable way of medication because thyroglobulin (or any iodinated tyrosine compound) acts only if it is converted into T4 by specific hydrolysis which is often faulty furthermore, there are cases on record of antibody formation against thyroglobuline during treatment with desiccated thyroid tablets (Godlowski 1965) Two forms of thyroid hormone medication have recently appeared on the market "Thyrolar ® (Armour Company) and Euthroid"® (Chilcott Warner Laboratory) Both forms contain combination of T3 with T4 in various proportions. The advantage of such combination is that one tablet contains both forms of thyroid hormone short and long acting The disadvantage, on the other hand, of such a combination is that the combination of the two forms of thyroid hormone eliminates the therapeutic maneuverability if one of them is not well tolerated or is not required

The dosage of T3 in adults over forty years is 5 μg three times daily if well tolerated and the larger quantities are required for the therapeutic effects, then a gradual step-up in dosage to the level of tolerance may be indicated In the same age group T4 is given along with T3 to prevent the suppressive ac

Table IV *Diagnosis of allergy*

Applied in the management of Menière's disease

	Diagnostic procedure	
1st day	Nonfasting patient	(a) History and physical examination (b) Cytotoxic food tests in a.m. (c) Inhalant skin tests. (1) vertical, (2) linear if required (d) Provocative skin tests in p.m. of positive Ag-foods found in cytotoxic tests and per history
2nd day	Fasting patient	( ) Cytotoxic food tests in a.m. (b) Metabolic panel with blood enzyme studies and X-ray of GI tract (c) Provocative skin testing of positive tests found in fasting cytotoxic tests
3rd day	After Ag-foods (feeding with positive foods and known from history in groups of five foods)	(a) Cytotoxic food tests of fed foods in a.m. (b) Provocative skin tests of fed foods in p.m.
4th day	After Ag-foods	Continuation if required as in 3rd day
5th day	After Ag-foods	Continuation if required as in 3rd day
6th day	Nonfasting	Résumé and outline of therapy

Ag-food. Food which causes positive reaction in one form of testing.

thereby initiate primarily or aggravate secondarily the existing pathology. Therefore the allergic reaction should be controlled whether it is the primarily precipitating factor in endolymphatic hypertension or whether it contributes secondarily.

The specific therapy of allergic reactions consist of the determination of the specific antigen and its injection in optimal dosage for hyposensitization, that is for the prevention of toxic proteolysis. The optimal dose is determined by skin titration (Rinkel, 1962) with the specific antigen in which the end-point constitutes the required initial dose. The details of the basic principles and technique of the therapy of allergic states are outlined in Parts V and VI of Volume 2 of *Allergy and Anaphylaxis as Metabolic Error* (Godlowski, 1965), and briefly summarized in Tables IV and V.

II. Symptomatic management of endolymphatic hypertension

Symptomatic therapy of endolymphatic hypertension consists in securing medical or surgical relief of symptoms derived from endolymphatic hypertension. Internists and surgeons who use these therapeutic methods claim no more than a control of the clinical manifestations of inner ear disease. Symptomatic relief of endolymphatic hypertension must, therefore, be regarded as an adjunct to the "etiological" therapy outlined in the previous paragraphs.

Symptomatic therapy of endolymphatic hypertension consists of (*a*) decompression of the endolymphatic hydrops, and (*b*) destruction of the inner ear.

Decompression of the endolymphatic hypertension in the management of endolymphatic hydrops

Decompression of the endolymph in hydrops may be accomplished either by pharmacologic or surgical means. Successful decompression assists in the recovery of the damaged inner ear organs. Correction of the dysproteinemia makes the beneficial effects of decompression more lasting. Therefore, *the two techniques should be applied concurrently in the rational management of endolymphatic hyperten-*

which participate in the activation of the enzymic reactions (as coenzymic factors) are also regarded as anabolic agents therefore their adequate supply orally or parenterally belong to the management of endolymphatic hypertension Mg Ca Mn Fe Fe and Co are best assimilated in the chelated form which guarantees their passage through the intestinal mucosa in an active form Minerals for oral administration should be complexed with ascorbic acid which acts as the ligand enteric coating of the tablets is effective in guaranteeing their passage through *stomach Such tablets are available on the* market as Hyalex®" and "Magnesium Plus®" (Pharmacal Company West Chicago Illinois)

D *Essential proteins supplement* In the management of endolymphatic hypertension a diet high in animal proteins will supply building material for the formation of the organisms own proteins. Essential proteins are recommended in amounts of 2 to 4 gm per kilo per 24 hours in forms of meat poultry fish milk and milk products, eggs, and the like These proteins however contain high animal fat which might be contraindicated in cases of endolymphatic hypertension associated with high blood cholesterol. In such an event the high protein diet should then be devoid of animal fats (well-done meats, skim milk egg whites, etc) or to administer "Questran® Meade Johnson (cholesteramin) which acts as a resin absorbing the dietary fats on the molecular surface (Godlowski 1965)

3 Hypothalamic suppression

Hypothalamic suppression by psychotropic medication oftentimes has a strategic value in the successful management of endolymphatic hypertension The hypothalamus is the central distributor of all stimuli penetrating the organism from the outside or generated within the body Stimuli which originate in various organs such as the liver gallbladder stomach, and duodenum might be projected to the inner ear and initiate inner ear manifestations such as vertigo (called sometimes vertigo stomachialis) tinnitus or nausea, thereby mimicking the symptoms of primary ear pathology Conversely primary ear diseases such as endolymphatic hypertension may be projected upon the subdiaphragmatic organs, and cause nausea and vomiting, which is also often associated with vertigo originated from the endolymphatic system.

Tranquilizing agents of a *central relaxant* and *hypothalamic suppressant* type block the redistribution of stimuli received from target organs. A central relaxant such as meprobamates or hypothalamic suppressants such as derivatives of phenothiazine and phenylmethane may be used concurrently with the other therapeutic agents included in the management of endolymphatic hypertension. For example there are readily available combination of anti-inflammatory steroid with hypothalamic suppressant such as "Ataraxoid® (Pfizer Laboratory) tablets containing either 2.5 or 5 mg of prednisone together with 10 mg of "Atarax® (hydroxizine HCl) The reader may find in the quoted references the details of administration of psychotropic drugs in reference Godlowski (1965)

4 Management of allergic reactions associated with endolymphatic hypertension

In our series the incidence of allergic reactions in patients with endolymphatic hypertension were around 67.2% (Table III) This need not necessarily be interpreted that the aural pathology is caused by hypersensitivity or that endolymphatic structures such as stria vascularis are directly involved in the allergic reactions, but hypersensitivity is, with little doubt one of the most common causes of endolymphatic hypertension The products of toxic proteolysis in the inner ear or elsewhere in the body which constitute the basic mechanism of allergic reaction (Godlowski 1962), may primarily or secondarily affect the depolymerizing action of hyaluronidase and influence the circulation of the endolymph

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Table V *Therapy of food allergy*

Applied to patient with Menière's disease with positive food allergy

1 Patients with food positive in all four groups of tests (+ + + +)
(a) Elimination of Ag-foods, next if controlled gradual rotation Ag-food with sublingual drops if digestive and/or metabolic error is controlled
(b) Digestive enzymes in large doses orally with each meal (supplemented with sublingual drops or subcutaneous injections if carrier or enzyme itself is made from animals positive in testing
(c) Correction of metabolic and/or organic error in GI tract
(d) Drops or subcutaneous injection of Ag-food according to result of skin titration

2. Patients with foods positive in three groups of tests (+ + +)
(a) Rotation of Ag-foods with supplementation of sublingual drops concurrently with therapy of digestive and/or metabolic error
(b) as in "1"
(c) as in "1"
(d) as in "1"

3 Patients with foods positive in two groups of tests (+ +)
(a) Sublingual drops of Ag-foods without rotation
(b) as in "1"
(c) as in "1"
(d) as in "1"

4 Patients with foods positive in one group of tests (+)
(a) Rotation diet of Ag-foods
(b) as in "1"
(c) as in "1"
(d) as in "1"

Adjustment of therapy according to tolerance and clinical results

This type of management presented in Tables IV and V is applied at present the 250 patients presented in the text were not managed in this way

sion as the ultimate etiological treatment one should not be substituted for the other If immuno-metabolic therapy combined with pharmacological decompression does not bring about complete or partial relief of clinical manifestations after twelve months of treatment, surgical decompression is in order *It must be strongly emphasized again that maintenance of immuno-metabolic therapy during and after successful surgical or pharmacological decompression is the only guarantee against a relapse of the disease* Discontinuation of the "etiologic" therapy of endolymphatic hydrops, if ever possible must be carried out stepwise and accompanied by laboratory studies of the abnormal features to be sure that these remain corrected. Decompressed patients when free or partially free of symptoms, are often unwilling to maintain the necessary metabolic therapy for many months, or for life. It must therefore, be impressed on these patients that they still harbour the insidious disease of dysproteinemia which may not only precipitate a recurrence of aural symptoms, but may also cause other syndromes derived from the same metabolic disease.

Pharmacologic decompression of endolymphatic hypertension

Lowering of the hyperosmolality of the endolymph can be accomplished by (1) administration of histamine or its analogues, (2) infusion of plasma proteins and/or plasma expanders to increase the relative osmolality of blood plasma.

Histamine hydrochloride or betahistine hydrochloride (Serc) *as decompressing agents of endolymphatic hypertension*

Histamine hydrochloride may be regarded as a hormone acting upon the microcirculation specifically on the precapillary constrictors (Schayer 1964) Histamine exists in all tissues in various quantities either in "free" form or "bound" to granules of the mast cells most likely connected with heparin (Schayer 1965). The "free" form of histamines may derive either from the "bound" form or may be de novo synthesized from histidine by decarboxylation this form of histamine Schayer (1965) called the induced histamine The "bound" form possesses no pharmacological action but many factors could release histamine (histamine liberators) thus making it "free" The degree of histamine effect is directly related to the quantity of "free" histamine. The phar

Table VI *Menière's disease and histamine cephalgia treated with pharmacological decompression*

	No. of Cases	Complete remission		Partial remission		No improvement		Deterioration	
		Abs.	%	Abs.	%	Abs.	%	Abs.	%
Menière disease	60	22	35.8	30	50.0	7	11.6	1	1.6
Histamine cephalgia	20	10	50.0	5	25.0	5	25.0	0	0

macological action of histamine has numerous effects, one of which is the relaxation of the precapillary constrictor leading to the substantial drop in blood pressure in the whole arterial system which may end, in advanced cases, in a vascular shock (Feldberg, 1953). The drop in the arterial head pressure in the precapillary system (in our case, in the *stria vascularis*) *may reduce the driving force* (see Fig. 1) of the filtration of water and water solutes from the capillaries into the extracapillary space (in our case, into the endolymphatic space). To accomplish this effect the dosage of histamine must reach the amounts which exhibits undesirable side-effects, e.g. wheal formation, exudation into various organs with manifestations from thus affected organs, etc. However histamine may bring about a transitory improvement in *Menière's disease* when applied sublingually or subcutaneously in an infinitesimally small amount, e.g., one to three drops of aqueous solutions ranging from 1 mg per million to 1 mg per 20 million. The effect of this therapy although harmless as far as side-effects of histamine is concerned, is unpredictable, and effective only in few cases of Menière's disease. Histamine administered orally is completely ineffective because it is destroyed by digestive enzymes.

Pharmaceutical industry has concentrated its efforts on formulating a histamine derivative which would retain its relaxing effect on the precapillary constrictor but be devoid of other undesired side-effects of histamine. *Betahistine hydrochloride* under the name of "Serc®" (Unimed, Inc., Morristown, New Jersey) has been studied from many pharmacological angles, and has been found to have an LD-50 about fifty times higher than that of histamine phosphate. Other side-effects are also reduced to an insignificant level, while its effect on relaxing of the precapillaries is fully retained but without the rapidity of the action exerted by histamine (Walter et al., 1941). We have, therefore, applied "Serc" orally in dosage 4 to 8 mg four times daily for decompression of endolymphatic hypertension (Godlowski, 1952). In this dosage, administered orally "Serc" is well tolerated in the majority of the cases of Menière's disease (Table VI). Only a few patients complained of general weakness caused by arterial hypotension this was easily remedied by oral administration of sympathomimetic drugs such as isoproterenol (Isuprel®) 15 mg three times daily. Hot flushes in the head occurred very rarely and responded to temporary discontinuation of "Serc" therapy with subsequent resumption at a lower dosage and gradually increasing to the recommended dose. On very rare occasions (less than 1%) higher doses of "Serc" could reactivate peptic ulcer hence in patients with active peptic ulcer or with history of it, this area should be carefully screened during the therapy with "Serc."

"Serc" has also been successfully used in other vascular pathologies such as histamine cephalgia (Horton & Van Leden, 1962), cerebrovascular senility syndrome (Horton & Van Leden, 1962), and angina pectoris (Horton & Van Leden, 1962).

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(a) Sublingual drops of Ag-foods without rotation

(b) as in 1

(c) as in "1

(d) as in "1"

4 Patients with foods positive in one group of tests (+)

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(b) as in "1"

(c) as in 1

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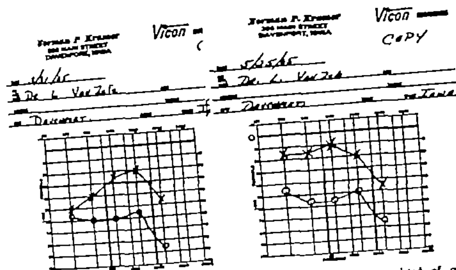


Fig. 6. Audiogram from patient with Menière's disease before and forty-eight hours after pharmacological decompression, the improvement in hearing measured seven weeks after decompression still persisted. Decompression consisted of oral administration of Serc 8 mg q.i.d. for six weeks prior to the infusion of 2 000 ml Osmitrol in course of four hours.

cologic decompression, no improvement occurs within four weeks, surgical decompression should be advised.

A transitory response to the intravenous infusion of plasma or plasma expanders may provide some information as to the probable success of surgical decompression in the improvement of hearing. A transitory effect in any clinical manifestations produced by increased osmolality of the blood indicates that the structures of the inner ear remain in a reversible state and thereby further surgical intervention is justified. In other words, a mild and transitory improvement may be regarded as a good prognostic sign as to the probable effectiveness of surgical decompression as far as hearing is concerned. However our experience in this respect is too limited to rule out surgical decompression in cases in which such transitory improvement did not occur

Supportive medical therapy in endolymphatic hypertension

Supportive therapy of endolymphatic hypertension consists of various measures which would improve the condition of the whole organism (Derlacki, 1965).

1 Complete *omission of tobacco* is a basic necessity in controlling endolymphatic hypertension in mild as well as in heavy smokers. The toxic effect of tobacco on the equilibral labyrinthine system has been conclusively established (Derlacki, 1954 and others) in clinical and in experimental conditions.

2 *Low-sodium diet* containing no more than 250 mg of sodium per day (Godlowski, 1965) has been recommended for reducing tissue hydration (in this instance the labyrinthine hypertension) The following sources of sodium should be kept in mind. table salt, and other dietary items which may have high content of the sodium cation, baking powder bicarbonate of soda, Accent," various condiments, carbonated drinks, drinking water from softeners, etc.

3. In acute disabling forms of endolymphatic hypertension *intravenous infusion of histamine phosphate* 1 to 2 mg in 500 ml of 5% dextrose solution in five hours (Derlacki, 1954).

4. In acute attacks of endolymphatic hypertension various compounds may have a bene-

The initial dosage of "Serc" may be gradually reduced to the maintenance level which must be individually determined by trial and error. The beneficial effects of betahistine hydrochloride therapy in Menière's disease must be regarded as a palliative procedure and should be used only as a supplement to the etiological therapy.

Increased osmolality of blood plasma used for decompression of endolymphatic hypertension

The return of water and crystalloids from the endolymph into the blood at the venous end of the stria vascularis is attracted by the osmotic pressure of plasma proteins and electrolytes (see Fig. 2). Therefore, increasing the osmolality of the plasma by means of infusion of the concentrated plasma proteins (or plasma expanders) should increase the rate of return of water from the endolymph to the blood thereby assisting the decompression of the endolymphatic hypertension. An intravenous infusion of concentrated blood plasma or concentrated human blood albumin (25%) is the most physiological material for increasing the osmolality of plasma. The concentrated proteins infused intravenously remain in the blood for about three to four weeks, and then the infusion should be repeated. The plasma protein preparations available on the market contain 25 g of albumin in 100 ml. To effectively influence the osmolality of the blood and decompress endolymphatic hypertension is around 1 000 to 2 000 ml of concentrated plasma (15 g proteins per 100 ml) or 250 to 500 g of proteins should be infused at one time. The dosage is calculated in the initial content of plasma proteins and their qualitative distribution. For example if the initial protein content of the patient's plasma was around 7 gm per 100 ml, the desired target is 10 gm percent of protein in the patient's plasma at the end of infusion. The hyperosmolality produced by about 10 gm per 100 of proteins in the blood plasma should effectively decompress the endolymphatic hypertension. There are two dangers in this method. (1) possibility of transmission of infection (hepatitis virus) from the pooled plasma. (2) a too rapid increase in blood volume caused by its hyperosmolality with the fluid withdrawn from the tissues into the blood.

Prospective patients must therefore possess (*a*) a normal electrolyte and water balance (*b*) satisfactory excretory capacity of kidneys, (*c*) an evaluated electrophoretic plasma protein distribution (*d*) a known osmolality of blood plasma and (*e*) a cardiovascular system in a fully compensated state.

Our practice is to mix 100 ml of the plasma expanders such as "Osmitrol®" "Albumisol®" or "Dextran®" with every 500 ml of plasma. Patients selected for pharmacological decompression should remain on the immuno-metabolic management for three to six months prior to the attempt and for at least three to six weeks on full dose of "Serc" therapy. An evaluation of protein distribution in plasma, the electrolyte profile, the osmotic pressure of blood plasma, the pH of blood and the viscosity of the plasma, should be done about twenty-four hours prior to an infusion and repeated immediately afterward, in order to be reasonably certain as to whether or not the required physicochemical conditions necessary for decompression have been fulfilled. A determination of total plasma proteins of ±10 g percent indicate that the infusion should be terminated. Some patients may experience transitory mild vertigo immediately after the infusion, so they should be informed about this beforehand.

Improvement in clinical manifestations may be expected within a few hours which, in unsuccessful decompression will shortly disappear. On the other hand, the improvement may not start to take place until two or three days after the infusion, but then be maintained for as long as the hyperosmolality of the plasma persists. During the whole post infusion period the patient must remain on full scale immuno-metabolic management. If after properly executed attempts of pharma

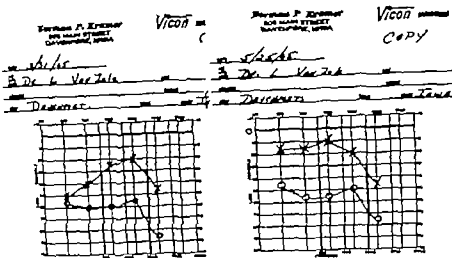


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Our practice is to mix 100 ml of the plasma expanders such as "Osmitrol®" "Albumisol®" or "Dextran®" with every 500 ml of plasma. Patients selected for pharmacological decompression should remain on the immuno-metabolic management for three to six months prior to the attempt and for at least, three to six weeks on full dose of Serc therapy An evaluation of protein distribution in plasma the electrolyte profile, the osmotic pressure of blood plasma the pH of blood, and the viscosity of the plasma, should be done about twenty-four hours prior to an infusion and repeated immediately afterward, in order to be reasonably certain as to whether or not the required physicochemical conditions necessary for decompression have been fulfilled. A determination of total plasma proteins of ±10 g percent indicate that the infusion should be terminated Some patients may experience transitory mild vertigo immediately after the infusion so they should be informed about this beforehand.

Improvement in clinical manifestations may be expected within a few hours which in unsuccessful decompression will shortly disappear On the other hand, the improvement may not start to take place until two or three days after the infusion, but then be maintained for as long as the hyperosmolality of the plasma persists. During the whole post infusion period the patient must remain on full-scale immuno-metabolic management. If after properly executed attempts of pharma

Table IX. *Effects of surgical decompression on clinical manifestation of Menière's syndrome*

Austin, et al., 1965

No. of cases over 70	Improved vertigo (%)	Improved tinnitus (%)	Improved hearing (%)	Improved discrimination (%)
	91	90	19	20

logical mechanism persisted in the organism even during the remission obtained by the decompression. In his cases the pre- and post operative metabolic studies were not reported.

R. J. Wolfson et al. (1968) applied cryosurgery to the horizontal semicircular canal chiefly for the relieving of vertigo of labyrinthine origin but disturbing the hearing only in very few patients.

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Average air conduction[a]	57.5	47.1
Average bone conduction	49.1	37.5
Average discrimination score[b]	62	71
Bekesy		
Type 1	1	
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Recruitment	7	1
Caloric response		
Normal	4	
Reduced	4	

[a] ASA standard.
[b] As measured by monitored live voice.

Table VII *Effects of surgical decompression on clinical manifestations of Menière's syndrome*

Portmann, 1964

No. of cases	Improved vertigo (%)	Improved tinnitus (%)	Improved hearing (%)
60	93	34.8	32

Table VIII *Effects of surgical decompression on clinical manifestations in Menière's syndrome*

House, 1964

Total = 63	Vertigo (%)	Tinnitus (%)	Hearing (%)	Pressure ( )
33 Positive				
Relief	47	11	0	35
Improved	29	40	11	64
No change	25	46	79	0
Worse	0	3	10	1

(In the vertigo column is a small statistical error — 101% total.)

ficial but inconsistent effect. "Antivert®" tablets (Roerig Company) 3 to 8 tablets per day are often helpful in controlling the annoying vertigo Bonine® (Pfizer Company) is recommended in 25 to 100 mg daily for protracted nausea and vomiting "Dramamine® (Searle Company) in tablets, suppositories and injections, in dosage of 50 to 500 mg may also occasionally interrupt the attack of vertigo. All these compounds possess antihistaminic effect so probably their alleviating action operates in vertigo connected with allergic reactions. Dilantine® (Parke Davis) 100 mg 1 to 3 capsules a day could be applied in protracted vertigos.

5 *Nicotinic acid* is a powerful vasodilating agent acting upon the arterioles and metarterioles and precapillary sphincter muscle of venules by this action the capillary circulation slows down and hydrostatic pressure drops down thereby reducing the rate of filtration in the stria vascularis and slowing production of endolymph. It also accelerates the reabsorption of water by the collecting venules thus reducing the volume of endolymph and improving the nutrition of the organs within the endolymphatic space. The effective dosage of nicotinic acid fluctuates between 500 to 8 000 mg per day Nicotinic acid, apart from a potent vasodilating effect possesses also important action in the metabolic process of protein formation.

Surgical decompression of endolymphatic hypertension

Surgical decompression of endolymphatic hypertension (if associated with hydrops) is attained by the formation of a fistula either (*a*) between the saccule and the vestibule or (*b*) between the endolymphatic sac and the surrounding structures containing free flowing fluids (subarachnoid space), or (*c*) by enlargement of the bony encasement in which the sac is located. The reader may find the details of each procedure in the quoted references.

G Portmann in 1926 and 1927 was the first to advocate fistulation of the endolymphatic sac to relieve endolymphatic hypertension but no attempt was made to secure permanent patency of the fistula. The results were remarkable for a short period of time, but two-thirds of the patients sooner or later relapsed. However by using a modification of the technique the results were highly successful in some patients over a long period (Portmann 1964)

W F House (1964) improved Portmann's technique by applying a shunt between the sac and subarachnoid space using a plastic prosthesis to keep the fistula open He (1968) has recently modified his initial operation by eliminating the prosthesis completely and has maintained an open fistula by applying cryo-otic-perotic shunt to the promontory thereby connecting the endolymphatic with perilymphatic spaces, thus equalizing the pressure in both compartments. His results chiefly in the relief of vertigo were somewhat better than those of Portmann. However when these technical devices failed, the episodes of vertigo promptly returned proving that the etio-

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Amman, et al., 1965

No. of cases over 70	Improved vertigo (%)	Improved tinnitus (%)	Improved hearing (%)	Improved discrimination (%)
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ASA standard.
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compression of the endolymphatic space although long-term observations have shown what appears to be a complete remission in some cases reported spontaneous remissions of five years duration must always be kept in mind in cases of therapeutic remissions. In these cases relief of the endolymphatic hypertension may have permitted the organism itself to correct the reversible aural pathology in some unknown manner especially if the existing metabolic error had a self limiting character However to hope for such an exceptional result and to routinely advise surgical decompression as the sole therapy in endolymphatic hypertension reflects an oversight in consideration of the concept of the biological process involved

Destructive surgery in the management of endolymphatic hypertension

Destruction of the inner ear should have a restricted application in the treatment of endolymphatic hypertension Destruction of the end organs in the inner ear may be accomplished either by a surgical procedure or by high frequency ultrasonic beam (Arslan, 1953 Ariano 1966) Both methods must be regarded as unphysiological, and as such, should be applied only to those patients who are incapacitated by vertigo and have a profound and probably irreversible hearing loss in the affected ear refractory to a prolonged and intensive immuno-metabolic therapy supplemented by medical and surgical decompressions.

Conclusions

1 Endolymphatic hypertension (Menière's Disease—Labyrinthine Hydrops) is a local expression of a generalized molecular disease of proteins which exists in the whole organism.

2 A deficiency in the depolymerizing action of hyaluronidase may lead to the accumulation of hydrophylic hyaluronate mucopolysaccharides in various parts of the organism, the specific location of the endolymphatic space in a narrow bone encasement may precipitate the clinical symptoms as a result of relatively mild hyperosmolality of the endolymph, whereas other organs involved in a similar disease and subjected to the same gradient of osmotic increase in the tissue fluids, do not respond with such dramatic manifestations as that of endolymphatic space.

3. The immuno-metabolic management of endolymphatic hypertension consists of the application of six therapeutic agents outlined in "The general principles of immuno-metabolic management" (Godlowski, 1965).

4. Conservative and/or surgical decompression of endolymphatic hypertension may facilitate the recovery of the aural disease, but immuno-metabolic management must be applied concurrently sometimes for life.

5 Decompression of endolymphatic hypertension may be attempted by either pharmacologic or surgical methods.

6. Both methods of endolymphatic decompression are mainly palliative procedures which fail to take into consideration the probable physiologic dysfunction that results in both pathological changes and the symptoms.

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My appreciation is also extended to Dr V Pullets, attending physician in the Ear Nose and Throat Department, Mayo Clinic, Rochester Minnesota, for sending the material used in my studies which would not have been available to an internist.

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GABRIEL MARSHAK, M.D., M.S.

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SUPPLEMENT 300

The Inner Ear in Experimental Diabetes Mellitus

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UPPSALA 1972

To my Parents

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I Introduction

Similar to other sensory receptors, the inner ear operates as a transducer in which the input signal serves as a trigger to initiate a transduction process energized by a metabolic source.

The discovery of the dc resting endolymphatic potential by Bekesy (1952) suggested the need to explore the metabolic pathways that controlled this phenomenon, since at that time it was already known that the other cochlear potentials were at least partially maintained by oxidative processes (Wever et al. 1941 Wever et al., 1949). Also the finding of high potassium levels in the endolymph (Smith et al., 1952: Smith et al. 1954), requiring powerful energy driven transport processes for its maintenance, re-emphasized the need for studies on the bioenergetics of the inner ear system.

The general concept was that the organ of Corti, due to its lack of capillaries and its remoteness from blood supply should have ample facilities for energy storage and anaerobic energy production the stria vascularis being a highly vascular structure, could probably depend more on oxidative metabolism and short term energy supply However because of the microscopic size, anatomic peculiarities of the tissues involved, and the difficulties encountered in obtaining and preparing the temporal bone, the studies pertinent to the energy metabolism of the cochlea have begun only recently

After the original histochemical demonstration, that the main energy supply to the brain is provided by oxidative glucose catabolism (Lowry 1953) Wing showed that in insulin-induced hypoglycemia, the cochlear microphonic was diminished quite markedly (Wing, 1959). Koide (1958), had shown that the reduced cochlear microphonics in insulin-induced hypoglycemia returned back to its normal values after injection of glucose or other citric acid cycle substrates. Two years later he demonstrated the adverse effect of various citric acid cycle inhibitors on the cochlear microphonics (Koide et al., 1960). Vosteen (1960) showed that the stria vascularis had high levels of enzymes for aerobic metabolism (such as succinic dehydrogenase, and those of the cytrochrome oxidase system), but had low levels of anaerobic metabolic enzymes such as lactic dehydrogenase. On the other hand, the organ of Corti had high levels of those same oxidative (aerobic) enzymes (not as high as the stria vascularis), but had high levels of lactic dehydrogenase (anaerobic) enzymes as well.

This chain of experiments, then shows that the inner ear is dependent on glucose metabolism, and that it is primarily aerobic.

With the refinement of bioelectric techniques, a more precise localization of cochlear events was possible. Thus, it was established that the cochlear microphonic has its origin in or near the hair cells, and the dc resting endolymphatic potential requires an intact stria vascularis for its existence (Bekesy 1951 Davis, 1956 Davis, 1958 Tasaki & Spyropoules, 1959: Davis, 1965 Wever 1966). With these techniques, it is possible now to examine in a dynamic way some of the metabolic processes involved in the inner ear function.

Diabetes mellitus is a chronic disorder of carbohydrate metabolism which is believed to result from relative or absolute insulin deficiency In addition to the severe metabolic disturbances, widespread pathological changes in the human body have been observed. These changes are basically vascular and consist of two types (Seftel & Walker 1966): nonspecific

large vessel disease and a specific small vessel disease or microangiopathy These changes are generalized throughout the body and indeed the few histological studies of temporal bones of human diabetics (Jørgensen 1961 Jørgensen 1963) and experimental animals (Costa 1967) show changes in the small blood vessels of the stria vascularis and the modiolus. These changes are apparently not specific but are more pronounced than those seen in "presbycusis" Sixteen to forty per cent of adult onset diabetes are found to audiometrically proven bilateral, symmetrical high frequency sensory neural hearing loss (Abu Khatwa et al., 1961 Zelenka & Kozak 1965 Ancona, 1956 Borsuk et al. 1956 Strubinski & Malicka 1966 Tota & Bocci, 1965 Vigi, 1950 Profatio & Baralelli, 1959 Marullo 1950 Jørgensen 1969 Jørgensen & Buch 1961) On the other hand juvenile onset diabetics are found to have normal pure tone audiograms, but show changes in the Bekesy tracings, the nature of which remain speculative (Marshak & Anderson 1968) The hearing loss associated with diabetes mellitus then may be a result of localized microangiopathy in the inner ear but it might also be due to derangement in the metabolism of glucose or a combination of the two

Recently the antibiotic streptozotocin derived from streptomyces Achromogenes was found to be diabetogenic (Vavra et al. 1959–1960 Herr et al. 1959–1960) Several studies have shown its specific destructive effect on the beta cells of the pancreas of experimental animals (Rableten et al. 1963 Brosky & Logathetopoulos, 1969) and of patients with functional islet cell carcinoma (Sado 1969) However the fact that no vascular changes have been observed using this drug (Arison et al., 1967) makes it an ideal tool to study the effect of pure and severe metabolic disturbance on the bioenergetics of the hearing processes.

PURPOSE

The purpose of this study was to examine the function of the inner ear of chinchillas suffering from chronic disturbance of glucose metabolism.

We elected to use chinchillas because of the stability of their hearing level over a long period of time. Long term experiments with the guinea pig are doomed because so many of them develop bony ankylosis and fixation of the ossicles. The intracochlear potential recording from the cat and other commonly used animals in auditory physiology is still fraught with great technical difficulties, in addition to the fact that their hearing level deteriorates with time

These chinchillas were rendered "diabetic" by partial selective medical pancreatectomy caused by the injection of streptozotocin. Cochlear function was assessed by studying the cochlear microphonic (CM) as an index of hair cell function and the dc resting endolymphatic potential (EP) as an index of the metabolic activity of the stria vascularis.

II Methods

1 Experimental animals. the study was performed on nine young healthy chinchillas, weighing 400–500 g ("Control" group) and 10 "diabetic" chinchillas of the same age and weight. Both groups were fed Alfalfa chinchilla diet and water ad libitum No insulin was administered.

2. Induction of the "diabetic" state was carried out by a single intraperitoneal injection of 100–120 mg/kg body weight of streptozotocin (NSC 37917) This state was maintained for 12–18 months prior to testing.

3 Drug formulation the drug was prepared aseptically as 1% solution in saline buffered

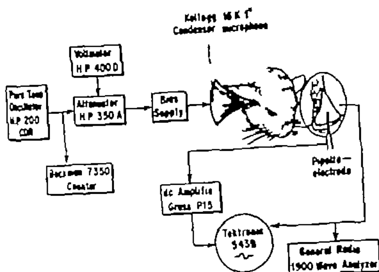


Fig. 1 Equipment used for delivering the signal and response recording.

with anticoagulant acid citrate dextrose solution (1 50 dilution A.C.D U.S.P with 0.9% sodium chloride). The pH of the solution was 5.0. The solutions were made fresh daily since the compound is very unstable. Freshly prepared solutions are crystal clear and have a light straw color On standing they take on a yellow to dark brown color and effervesce—both changes are indicative of instability of the compound.

4. Monitoring of the diabetes was carried out by checking for glycosuria twice a week with clinistix

5 Blood sugar was determined by the alkaline potassium ferricyanide method with Technicon Autoanalizer modification (Hoffman, 1937)

6 Surgical procedure. with the chinchilla anesthetized and supine the head was held in a custom-designed head holder A 3 cm incision through skin and platysma parallel to the body of the mandible was carried out. By sharp and blunt dissection the bulla was exposed after the overlying muscle was divided between ligatures. The bulla was entered using a mm cutting dental burr or exavators and the opening was enlarged with rongeurs. The mucosa over the elongated cylindrically-shaped cochlea was stripped with a straight ear pick so as to bare the basal and the second turn. Using a 1 mm polishing burr the lateral aspect of the otic capsule of the basal turn was flattened slowly until the blue line" was seen. A perforation through the bone was done by manual twisting of a root canal reamer 0.1 mm tip diameter The wire electrode fitted snugly into the hole was cemented to the margins of the cochlea with Caulk grip cement.

For the endolymphatic dc potential recording, either the same hole was utilized or another hole was drilled directly over the spiral ligament of the same ear or the contralateral one.

7 Electrodes:

(*a*) For the CM recording, 100 micron Teflon coated nonpolarizable nichrome wire was used.

(*b*) For the dc recording, micropipettes were pulled by Automatic Vertical Pipette Puller (David Kopf Instruments, model 700B) from pyrex glass tubing of approximately 1 mm in its external diameter The external tip diameter of the micropipette was 3–5 micron with tip resistance of 5–15 megohms.

By placing these micropipettes tip down, in

a 3 molar KCL bath thus solution passively diffused to partially fill the pipette An occasional air bubble was displaced with minute wire.

(*c*) The micropipette was connected to the amplifier with a chloridized silver wire

Chloridization was carried out at the anode in a bath containing 0 1 M Hydrochloric acid. A potential difference of 2 or 3 volts between bath and electrode was maintained for 30 minutes.

8 Instrumentation and Measurements the signal system consisted of pure tone oscillator (Hewlett Packard 200 CDR) attenuator (Hewlett Packard 350A) and a 200 volt bias supply (custom made) which was connected to a Kellog model 16K 1 condensor microphone. Short pips of pure tone from 500 Hz to 4 000 Hz that were produced by the oscillator the frequency of which was monitored by a Beckman 7350 counter were coupled to the partially excised external auditory canal of the chinchilla through a modified ear speculum.

The potential differences between the intracochlear electrode and an indifferent electrode in the neck muscles were measured with a General Radio 1900 wave analyzer and at the same time were seen on a Tektronix 543B oscilloscope.

The dc potential recorded from the scala media was amplified 100 × by dc amplifier (Grass P 15) and measured on the oscilloscope (Fig 1) In this manner the correct position of the exploring micropipette could be ascertained.

At the end of the procedure the animals were sacrificed and biopsies from the liver kidney spleen and pancreas were obtained. Microscopic sections were cut at 12 microns and stained with hematoxylin and eosine.

III Experimental Design and Procedure

Normal and diabetic" chinchillas were studied on alternate days, and were kept fasting over night Because of the difficulty in obtaining blood samples, only one sample from each animal was submitted for sugar determination. This was obtained by excising the tip of the tail immediately after the animal was anesthetized.

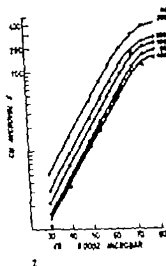
Recording of the CM was then carried out in a duobly walled sound treated room (Industrial Acoustics Company Inc)

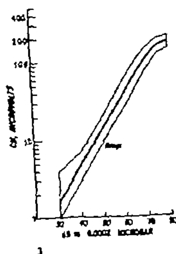
Measurements of the CM for each animal for each frequency were obtained twice first when the signal intensity was gradually increased to reach the peak response the second measurement was taken while gradually decreasing the signal intensity

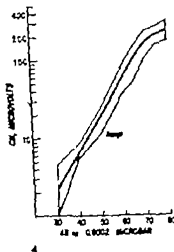
The recording of the CM was carried out from one ear and the dc potential from the other ear either by penetrating the spiral ligament or through Reissner s membrane.

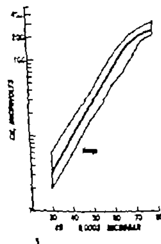
Table I *Fasting blood sugar levels in normal and diabetic chinchillas*

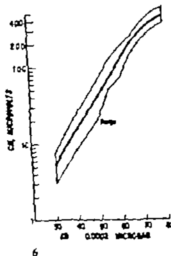
Chinchilla no.	Normal chinchilla (mg)	Chinchilla no.	Diabetic chinchilla (mg ~)
1	110	14	709
	110	16	409
4	115	17	350
5	110	18	380
6	115	20	303
8	110	1	320
10	105	23	270
11	115	24	320
26	115	28	350
		30	370











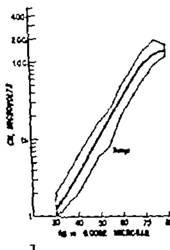


Fig 2 Mean response of 9 normal chinchillas to all frequencies tested
Fig 3 Response of 9 normal chinchillas at 500 Hz.
Fig 4 Response of 9 normal chinchillas at 1 000 Hz
Fig 5 Response of 9 normal chinchillas at 2 000 Hz.
Fig 6. Response of 9 normal chinchillas at 3 000 Hz.
Fig 7 Response of 9 normal chinchillas at 4 000 Hz.

IV Results and Discussion

All the chinchillas that were injected with streptozotocin 100–120 *mg* kg body weight developed glycosuria within 4 hours, which persisted for 17 18 months. No acetonuria was detected.

Table I shows the fasting blood sugar values for the diabetic and control groups, 12 18 months after the induction of the "diabetic" state. There is a definite and consistent hyperglycemia in the diabetic" group. These chinchillas did not require insulin and enjoyed excellent health.

Extensive histological survey of the liver kidney pancreas and spleen did not show any

a 3 molar KCL bath this solution passively diffused to partially fill the pipette. An occasional air bubble was displaced with minute wire.

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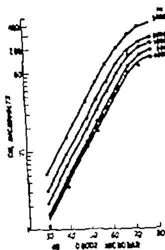
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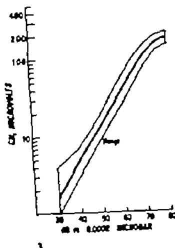
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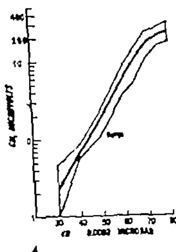
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Chinchilla no.	Normal chinchilla (mg)	Chinchilla no.	Diabetic chinchilla (mg)
1	110	14	[illegible]09
2	110	16	409
4	115	17	350
5	110	18	380
6	115	20	303
8	110	21	320
10	105	[illegible]3	[illegible]70
11	115	[illegible]4	3[illegible]0
[illegible]6	115	[illegible]8	350
		30	370

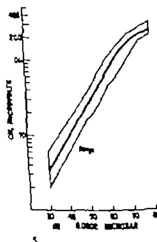




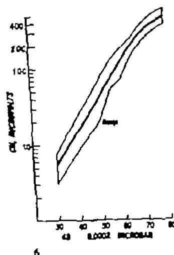
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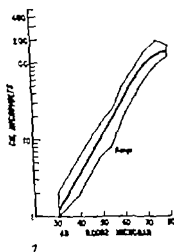
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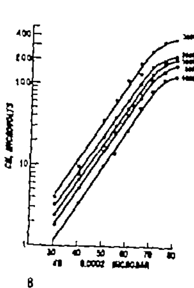
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IV Results and Discussion

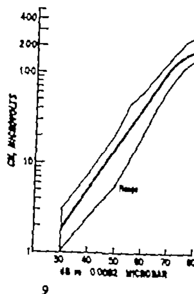
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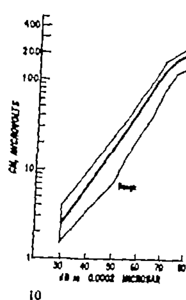
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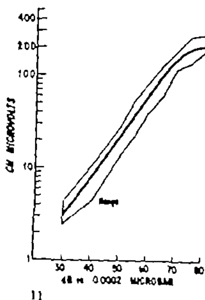
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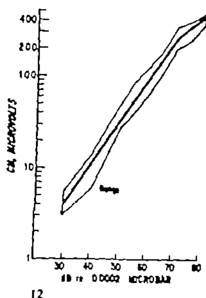
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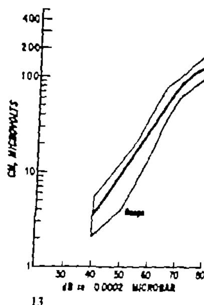
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12



13

Fig 8 Mean response of 10 diabetic chinchillas in all frequencies tested.
Fig 9 Response of 10 diabetic chinchillas at 500 Hz.
Fig 10 Response of 10 diabetic chinchillas at 1 000 Hz.

Fig 11 Response of 10 diabetic chinchillas at 2 000 Hz.
Fig 12 Response of 10 diabetic chinchillas at 3 000 Hz.
Fig 13 Response of 10 diabetic chinchillas at 4 000 Hz.

evidence of vascular changes, neither in the large nor in the small vessels.

Fig 2 shows the mean response of the 9 normal ("control") chinchillas in all frequencies tested Figs. 3 4 5 6 and 7 showed the intensity function of the same group at 500 Hz, 1 000 Hz, 2 000 Hz, 3 000 Hz, and 4 000 Hz, respectively

Fig 8 shows the mean response of the 10 "diabetic" chinchillas in all frequencies tested,

Table II *dc resting endolymphatic potential of normal and diabetic chinchillas*

Chinchilla no.	Normal chinchillas (mV)	Chinchilla no	Diabetic chinchillas (mV)
6	70	14	75
10	80	16	85
11	80[a]	17	70[a]
8	70	24	70

[a] Scala media entered through Reissner's membrane.

Table III. *Differences between the response of normal and diabetic chinchillas at 500 Hz*

Signal intensity in dB re 0.0002 microbar	Normal chinchillas Mean (microvolt)	Normal chinchillas S.D	Diabetic chinchillas Mean (microvolt)	Diabetic chinchillas S.D	t (df = 17)	p
30	1.6	1.0	1.8	1.0	0.5320	NS
40	4.5	1.0	5.5	1.0	0.6632	NS
50	13.0	4.0	15.0	5.0	0.5827	NS
55	24.0	7.0	25.0	5.0	0.2390	NS
60	36.0	11.0	40.0	6.0	0.9453	NS
65	66.0	6.0	68.0	4.0	0.0261	NS
70	113.0	11.0	114.0	13.0	0.055	NS
75	154.0	15.0	146.0	11.0	0.0987	NS
80	172.0	12.0	177.0	12.0	0.3082	NS

whereas the response of this group to 500 Hz, 1 000 Hz, 2 000 Hz, 3 000 Hz, and 4 000 Hz is shown in Fig. 9 10, 11 12 and 13 respectively

The magnitude of the dc endolymphatic potential was measured in four chinchillas in each group as seen in Table II. In three chinchillas in each group, the electrode was introduced to the scala media through the spiral ligament, in one chinchilla in each group the micropipette was pushed through Reissner's membrane. By manipulating the micromanipulator back and forth, after entering the scala media, three measurements were obtained for each animal.

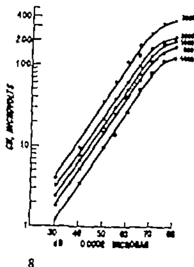
While monitoring the passage of the micropipette through the spiral ligament and stria vascularis, the dc potential was never more than a few millivolts, and occasionally was even negative, for a brief period of time, relative to the inactive electrode placed in the neck muscle.

However when the electrode was pushed further inward, there suddenly appeared a large positive potential, of 70–85 millivolts in magnitude, which remained unchanged as long as the electrode stayed in that position. At this point, there was always an increase in the microphonic response picked up by the same electrode up to 2–5 millivolts.

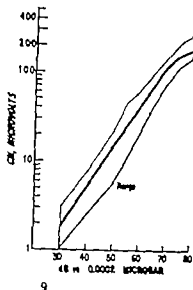
The statistical analysis of the responses of the two groups at each frequency tested is shown in Tables 3, 4 5 6, and 7 The similarity between the functions of the two groups is striking, both in terms of the maxima of the responses and in terms of the slope of the curves. Indeed, the difference between the two groups is insignificant except for their

Table IV *Differences between the response of normal and diabetic chinchillas at 1000 Hz*

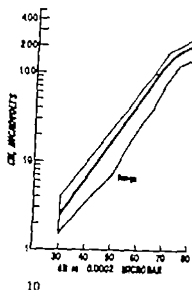
Signal intensity in dB re 0.0002 microbar	Normal chinchillas Mean (microvolts)	Normal chinchillas S.D	Diabetic chinchillas Mean (microvolts)	Diabetic chinchillas S.D	t (df = 17)	p
30	2.5	1.0	2.5	1.0	0.641	NS
40	6.5	1.0	6.5	2.0	0.1091	NS
50	19.0	4.0	18.0	5.0	0.6153	NS
55	32.0	7.0	30.0	4.0	0.5733	NS
60	57.0	6.0	30.0	9.0	0.7806	NS
65	97.0	10.0	80.0	10.0	1.3547	NS
70	167.0	12.0	134.0	11.0	1.9566	0.10
75	190.0	14.0	179.0	16.0	0.8008	NS
80	215.0	19.0	204.0	19.0	0.5564	NS



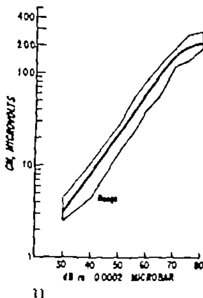
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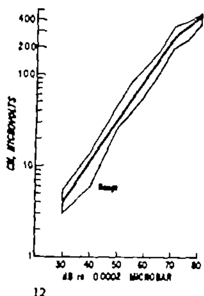
9



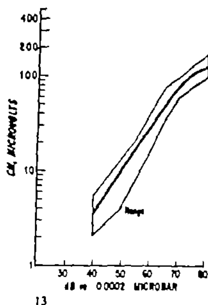
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Fig 8 Mean response of 10 diabetic chinchillas in all frequencies tested.
Fig 9 Response of 10 diabetic chinchillas at 500 Hz.
Fig 10 Response of 10 diabetic chinchillas at 1 000 Hz.
Fig 11 Response of 10 diabetic chinchillas at 2 000 Hz.
Fig 12 Response of 10 diabetic chinchillas at 3 000 Hz.
Fig 13 Response of 10 diabetic chinchillas at 4 000 Hz.

evidence of vascular changes, neither in the large nor in the small vessels.

Fig. 2 shows the mean response of the 9 normal ("control") chinchillas in all frequencies tested. *Figs.* 3 4 5 6, and 7 showed the intensity function of the same group at 500 Hz, 1 000 Hz, 2 000 Hz, 3 000 Hz, and 4 000 Hz, respectively

Fig. 8 shows the mean response of the 10 "diabetic" chinchillas in all frequencies tested,

Table II *dc resting endolymphatic potential of normal and diabetic chinchillas*

Chinchilla no.	Normal chinchillas (mV)	Chinchilla no	Diabetic chinchillas (mV)
6	70	14	75
10	80	16	85
11	80*	17	70*
8	70	4	70

Scala media entered throug [illegible]

Table III. *Differences between the response of normal and diabetic chinchillas at 500 Hz*

Signal intensity in dB re 0.0002 microbar	Normal chinchillas		Diabetic chinchillas			
	Mean (microvolts)	S.D.	Mean (microvolts)	S.D.	t (df = 17)	p
30	1.6	1.0	1.8	1.0	0.5320	NS
40	4.5	1.0	5.5	1.0	0.6632	NS
50	13.0	4.0	15.0	5.0	0.5827	NS
55	24.0	7.0	25.0	5.0	0.2380	NS
60	36.0	11.0	40.0	6.0	0.9433	NS
65	66.0	6.0	68.0	4.0	0.0263	NS
70	113.0	11.0	114.0	13.0	0.055	NS
75	154.0	15.0	146.0	11.0	0.0987	NS
80	172.0	12.0	177.0	12.0	0.3082	NS

whereas the response of this group to 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, and 4000 Hz is shown in Fig. 9, 10, 11, 12 and 13 respectively.

The magnitude of the dc endolymphatic potential was measured in four chinchillas in each group as seen in Table II. In three chinchillas in each group, the electrode was introduced to the scala media through the spiral ligament. In one chinchilla in each group the micropipette was pushed through Reissner's membrane. By manipulating the micromanipulator back and forth, after entering the scala media, three measurements were obtained for each animal.

While monitoring the passage of the micropipette through the spiral ligament and stria vascularis, the dc potential was never more than a few millivolts, and occasionally was even negative, for a brief period of time, relative to the inactive electrode placed in the neck muscle.

However, when the electrode was pushed farther inward, there suddenly appeared a large positive potential, of 70–85 millivolts in magnitude, which remained unchanged as long as the electrode stayed in that position. At this point, there was always an increase in the microphonic response picked up by the same electrode up to 2–5 millivolts.

The statistical analysis of the responses of the two groups at each frequency tested is shown in Tables 3, 4, 5, 6, and 7. The similarity between the functions of the two groups is striking, both in terms of the maxima of the responses and in terms of the slope of the curves. Indeed, the difference between the two groups is insignificant except for their

Table IV. *Differences between the response of normal and diabetic chinchillas at 1000 Hz*

Signal intensity in dB re 0.0002 microbar	Normal chinchillas		Diabetic chinchillas			
	Mean (microvolts)	S.D.	Mean (microvolts)	S.D.	t (df = 17)	p
30	2.5	1.0	2.5	1.0	0.641	NS
40	6.5	1.0	6.5	2.0	0.1091	NS
50	19.0	6.0	18.0	3.0	0.6153	NS
55	32.0	7.0	30.0	4.0	0.3733	NS
60	57.0	6.0	50.0	9.0	0.7806	NS
65	97.0	10.0	80.0	10.0	1.3547	NS
70	167.0	12.0	134.0	11.0	1.9566	0.10
75	190.0	14.0	179.0	16.0	0.8008	NS
80	215.0	19.0	204.0	19.0	0.5564	NS

Table V *Differences between the response of normal and diabetic chinchillas at 2000 H*

Signal intensity in dB re 0 0002 microbar	Normal chinchillas		Diabetic chinchillas			
	Mean (microvolts)	S.D	Mean (microvolts)	S.D	t (df = 17)	p
30	3.0	1.5	3.5	0.5	0,2030	NS
40	9 0	3.5	8.0	1.0	1 0623	NS
50	26.0	7 0	22.0	4 0	1.5134	NS
55	44 0	7 0	38 0	10 0	0.9905	NS
60	74 0	9 0	6_0	10 0	1.50_	NS
65	126.0	9 0	100.0	10.0	0.7270	NS
70	181 0	12.0	162.0	15 0	0 682_	NS
75	225 0	19 0	200.0	19 0	1.3164	NS
80	_50.0	17 0	220.0	19 0	1.5985	NS

responses at 3 000 Hz tone at 30 dB and 40 dB which may reflect a recording error or could have happened by chance alone.

The only study of cochlear microphonics in normal chinchillas was reported by Strother in 1967 (Strother 1967) His active electrode was placed on the round window membrane. Since there are no differences between the diabetic" and the control group in our study the 19 chinchillas can be treated as one group indeed, except for a slight decrease in the maxima of the response in all frequencies tested, Strother s curves and ours compare favorably in terms of the slope and the linear and non linear portions of the function

The values for the dc endolymphatic potential in our group—70–85 millivolts—is of the same magnitude as was reported for cats, guinea pigs monkeys (Fernandez et al. 1962) as well as for chinchillas (Eldredge 1968)

Several factors could account for the fact that the cochlear potentials were not affected in our "diabetic" group

1 The role of insulin in glucose metabolism is still debatable. However the majority of investigators share the opinion that it facilitates the penetration of glucose into the cell to be utilized for energy production or to be stored as glycogen (*Cecil Loeb Textbook of Medicine* 1963) This phenomenon has been demonstrated in many tissues, except in the brain and in the liver where glucose utilization is not insulin dependent Since the cochlea is a neuroepithelial structure, it is possible that its glucose metabolism is insulin independent. In this case, in spite of the fact that insulin production was partially or totally curtailed (as in our diabetic" group) the glucose-dependent hearing processes will not be affected.

Table VI *Differences between the response of normal and diabetic chinchillas at 300 H*

Signal intensity in dB re 0 000_ microbar	Normal chinchillas		Diabetic chinchillas			
	Mean (microvolts)	S D	Mean (microvolts)	S.D	t (df 17)	p
30	5 0	1 5	4 0	1 0	1 9230	0.10
40	15 0	3.5	10.0	_0	_9834	0.01
50	43 0	7 0	36.0	3.5	1 3090	NS
55	74 0	7 0	65 0	8.0	0 9180	NS
60	122.0	9 0	110.0	10.0	0.8803	NS
65	200.0	12.0	182.0	12.0	0 9111	NS
70	272.0	20 0	65.0	18.0	1.454	NS
75	355 0	30 0	337 0	29 0	1 195	NS
80	450.0	30.0	430.0	34 0	1 3745	NS

Table VII. *Differences between the response of normal and diabetic chinchillas at 4000 Hz*

Signal intensity in dB re 0.0002 microbar	Normal chinchillas		Diabetic chinchillas			
	Mean (microvolts)	S.D.	Mean (microvolts)	S.D	*t* (df = 17)	*p*
30	Could not be measured		Could not be measured			
40	3.0	1.5	3.5	1.5	0.4576	NS
50	11.0	3.0	10.0	3.0	1.0967	NS
55	18.0	4.0	14.0	4.0	1.228	NS
60	34.0	6.0	26.9	5.0	1.2603	NS
65	42.0	9.0	51.0	7.0	0.336	NS
70	96.0	9.0	82.0	6.0	1.5041	NS
75	115.0	12.0	115.0	10.0	1.0977	NS
80	141.0	12.0	125.0	10.0	1.2377	NS

2. After this study was started, a method of chemical microanalysis of individual cell types of the cochlea was developed (Matschinsky et al. 1968). The importance of this method is in the fact that the analysis is carried out without alteration of metabolic pattern: this is achieved by rapid freezing of the cochlea and analyzing microsamples of individual cell types. Using this method, Matschinsky & Thalman found (1967, 1970) that the glycogen levels in the organ of Corti of the guinea pig are very high—approximately ten fold higher than demonstrated in the average brain but the same order of magnitude found in the average rabbit retina. The amount of glycogen in the stria vascularis was considerably less. Of interest is the fact that the main localization of this glycogen stores is in the external hair cells. However, in addition to this large amount of stored energy, a high level of glucose was found in the organ of Corti. It was found to be located largely in the extracellular space but is readily available for immediate use, or when the stored glycogen is depleted—a situation which might theoretically exist in the hypoinsulinemic chinchilla.

3. The general angiopathy might involve the central auditory pathways (Reske-Nielsen et al. 1965, Dolman, 1963, Fagerberg, 1959) and cause a hearing loss which will not be manifested by changes in the cochlear potentials.

V. Summary and Conclusions

Sustained hyperglycemia without generalized microangiopathy was induced in 9 chinchillas and maintained for 12–18 months by a single intraperitoneal injection of streptozotocin 100–120 mg/kg body weight.

Inner ear function in these chinchillas measured by unipolar intracochlear recording of the cochlear microphonics and the dc resting endolymphatic potential was found to be normal.

The hearing loss found in human diabetics might possibly be due to the generalized angiopathy affecting the capillaries of the basilar membrane. These capillaries are supplied by different routes than those supplying the stria vascularis (Axelsson, 1968). The role of these capillaries as providing the organ of Corti with oxygen and nutrients may be far more important than generally realized (Lawrence, 1966). Indeed, the hearing loss caused by noise stimulation (Lawrence et al. 1967) and ototoxic drugs (Hawkins, 1967, Lawrence, 1970) may be primarily due to damage to these capillaries. Thus, the absence of angio-

pathy in the injected group of chinchillas could certainly explain the normal cochlear potentials by the same token the etiology of the hearing loss found in human diabetics might possibly be explained on the basis of the diabetic microangiopathy affecting the capillaries of the basilar membrane (vas spirale)

VI Acknowledgment

I would like to extend my gratitude to my thesis supervisor Dr A. M Small, Jr His patience, support and encouragement were indispensable during the entire project.

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